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# Health Assessment Document for Polychlorinated Dibenzo-p-Dioxins

(U.S.) Environmental Protection Agency
Cincinnati, OH

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15. SUPPLEMENTARY NOTES

16. ABSTRACT

The health assessment document on polychlorinated dibenzo-p-dioxins discusses multimedia environmental issues pertaining to the most toxic chlorinated dioxins, namely, 2,3,7,8-tetrachloro-; 1,2,3,7,8-pentachloro-; 1,2,3,6,7,8-hexachloro-; and 1,2,3,7,8,9-hexachlorodibenzo-p-dioxins. Scientifically valid data essential for human health risk assessment purposes from an extensive literature search have been compiled and discussed critically. Discussions are based on physiochemical properties and analytical methodologies; stability and degradation; production, use, synthesis; environmental resources and environmental levels; environmental fate and transport; degradation; bioaccumulation and bioconcentration factors; ecological effects; various aspects of toxic effects from acute, subchronic and chronic exposures in experimental animals and humans; pharmacokinetics and mechanism of toxic effects; teratogenicity and reproductive effects; mutagenicity and carcinogenecity. Based on this review, critical studies have been identified and utilized for estimating the unit risk.

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# Health Assessment Document for Polychlorinated Dibenzo-p-Dioxins

U.S. ENVIRONMENTAL PROTECTION AGENCY
Office of Research and Development
Office of Health and Environmental Assessment
Environmental Criteria and Assessment Office
Cincinnati, Ohio 45268

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## NOTICE

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsment or recommendation for use.

# PREFACE

The Office of Health and Environmental Assessment has prepared this Health Assessment Document on polychlorinated dibenzo-p-dioxins at the request of the Office of Air Quality Planning and Standards.

In the development of this assessment document, the scientific literature has been inventoried, key studies have been evaluated, and summary and conclusions have been prepared such that the toxicity of polychlorinated dibenzo-p-dioxins is qualitatively and where possible, quantitatively, identified. Observed effect levels and dose-response relationships are discussed where appropriate in order to identify the critical effect and to place adverse health responses in perspective with observed environmental levels.

This document was reviewed by a panel of expert scientists during the peer review workshop held at the Cincinnati Convention/Exposition Center, Cincinnati, OH, on July 27, 26 and 29, 1983. The Environmental Health Committee and the Environmental Effects, Fate and Transport Committee of the U.S. EPA's Science Advisory Board independentally reviewed the document in a public session.

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| ADI | Acceptable daily intake |
| AHH | Aryl hydroxycarbon hydroxylase |
| bw | Body weight |
| BCF | Bioconcentration factor |
| BromoPeCDD | Bromopentachlorodibenzo-p-dioxin |
| DCDD | Dichlorodibenzo-p-dioxin |
| DMSO | Dimethylsulfoxide |
| DNA | Deoxyribonucleic acid |
| EC/GC | Electron capture/gas chromatography |
| $ED_{50}$ | Median effective dose |
| FEL | Frank effect level |
| GC/MS | Gas chromatography/mass spectrometry |
| GC/SIM/MS | Gas chromatography/specific ion monitoring/mass spectrometry |
| HPLC | High performance liquid chromatography |
| HRGC | High resolution gas chromatography |
| HRMS | High resolution mass spectrometry |
| HxCDDs | Hexachloro derivatives of dibenzo-p-dioxins |
| $LC_{50}$ | Concentration lethal to 50% of recipients |
| $LD_{50}$ | Dose lethal to 50% of recipients |
| LOAEL | Lowest-observed-adverse-effect level |
| LRMS | Low resolution mass spectrometry |
| MFO | Mixed function oxidase |
| NICI | Negative ion chemical ionization |
| NOAEL | No-observed-adverse-effect level |
| NOEL | No-observed-effect level |

## LIST OF ABBREVIATIONS (cont.)

| | |
|---|---|
| OCDD | Octachlorinated dibenzo-p-dioxins |
| PCDDs | All polychlorinated dibenzo-p-dioxins |
| PCP | Pentachlorophenol |
| PeCDDs | Pentachloro derivatives of dibenzo-p-dioxins |
| ppb | Parts per billion |
| ppm | Parts per million |
| ppt | Parts per trillion |
| RBC | Red blood cells |
| RNA | Ribonucleic acid |
| SA | Satellite association |
| TCDDs | Tetrachloro derivatives of dibenzo-p-dioxins |
| TriCDD | Trichlorodibenzo-p-dioxin |
| 2,4,5-T | 2,4,5-Trichlorophenoxyacetic acid |
| TWA | Time-weighted average |
| UV | Ultraviolet |
| WCOT | Wall-coated open tubular |

# 1. INTRODUCTION

Dioxins are a class of compounds that contain the dibenzo-*p*-dioxin nucleus. In chlorinated dioxins, the dibenzo-*p*-dioxin nucleus is substituted with chlorine at different positions of the fused benzene rings. Depending on the number and position of chlorine substitution, 75 congeners are possible for the chlorinated dioxins. This document deals with the most toxic chlorinated dioxins, namely, 2,3,7,8-tetrachloro-, 1,2,3,7,8-pentachloro-, 1,2,3,6,7,8-hexachloro- and 1,2,3,7,8,9-hexachlorodibenzo-*p*-dioxin. Of these four congeners, the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin has been studied extensively and is often described in both popular and technical literature as "TCDD" or simply "dioxin."

A few documents exists at the present time that deal with selected aspects of polychlorinated dibenzo-*p*-dioxins in the environmental media. This document, however, has been prepared to provide a comprehensive multimedia assessment of the analytical methodologies, environmental levels and ecological and health effects of the four chlorinated dioxins. The following acronyms will hereafter be used when discussing the polychlorinated dibenzo-*p*-dioxins:

| | |
|---|---|
| PCDDs | Polychlorinated dibenzo-*p*-dioxins |
| 2,3,7,8-TCDD | 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin |
| 1,2,3,7,8-PeCDD | 1,2,3,7,8-Pentachlorodibenzo-*p*-dioxin |
| 1,2,3,6,7,8-HxCDD | 1,2,3,6,7,8-Hexachlorodibenzo-*p*-dioxin |
| 1,2,3,7,8,9-HxCDD | 1,2,3,7,8,9-Hexachlorodibenzo-*p*-dioxin |

# 2. SUMMARY AND CONCLUSIONS

## 2.1. SUMMARY

Polychlorinated dibenzo-p-dioxins are a class of chlorinated tricyclic aromatic hydrocarbons consisting of two benzene rings connected by a pair of oxygen atoms. According to the position and number of chlorine atoms it is possible to form 75 different congeners of chlorinated dioxins. The word "dioxins" is often used to refer to this class of compounds, especially with respect to the highly toxic and environmentally widely distributed 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). This class of compounds is rather stable toward heat, acids and alkalis. The solubility of 2,3,7,8-TCDD in water is 0.2 μg/ℓ. This isomer and the three other PCDDs discussed in this document are soluble in certain aromatic and aliphatic solvents. The PCDDs are chemically relatively stable and start to decompose at temperatures >500°C; the percent of decomposition depends upon the residence time in the high temperature zone and the proportion of oxygen in the heated zone.

The commonly used method for the determination of these compounds in different samples consists of solvent extraction, followed by sulfuric acid and base washes to remove lipids and other impurities from the solvent extract. The extract is then subjected to two liquid chromatographic clean-up procedures. The cleaned-up extract is finally analyzed for the PCDDs by the gas chromatographic-mass spectrometric methods. Despite the specialized methods used for the determination of PCDDs, the results of analysis at very low levels (possibly <9 ppt in biological matrices) can be questionable unless special precautions, including addition of internal standard, are made.

None of the PCDDs are either commercially manufactured or have any known use. They are produced as unwanted contaminants primarily during the manufacture of chlorophenols and their derivatives. The primary sources of PCDD contamination in the environment result from the industrial manufacture of chlorophenols and their derivatives and the subsequent disposal of wastes from these industries. Municipal incineration may also produce some environmental emission of PCDDs. From the available data, it is difficult to ascertain the comparative importance of these three sources in contributing to environmental emissions. The 1,2,3,7,8-PeCDD found in environmental samples has only been reported in emissions from incinerators.

The monitoring data to date indicate that the maximum level of PCDDs is likely to be found in soil and drainage sediment samples near chlorophenol manufacturing industries and chemical waste disposal sites. With the exception of air near certain contaminated sites, only very limited attempts have been made to determine the level of PCDDs in air samples. In the United States, the highest levels are reported at certain hazardous waste sites and in fish and wildlife tissue from areas contaminated with 2,3,7,8-TCDD.

The environmental fates of the four PCDDs are not known with certainty. Most of the investigations in this field have been conducted with 2,3,7,8-TCDD, and the conclusions regarding the environmental fate of the other three PCDDs have been drawn by analogy. Few data exist in the literature that would indicate significant chemical and biological transformation of these compounds in atmospheric, aquatic or soil media. The role of photochemical transformation in determining the fates of these chemicals in various ambient media is not known with certainty, but the PCDDs are susceptible to photochemical reactions in the presence of hydrogen donors. In the aquatic media, a substantial proportion of the PCDDs may be present in the sediment-sorbed state or in the biota. In the atmosphere, the PCDDs are expected to be present in the vapor-phase and particulate-sorbed states.

The atmospheric transport of these compounds can be predicted from dispersion modeling equations. In the case of the accidental release of 2,3,7,8-TCDD at Seveso, Italy, it has been estimated from laboratory experiments that 2,3,7,8-TCDD deposition from air to soil follows an exponential decay pattern along the downward wind direction. The most probable transport mechanisms of the PCDDs from soils are transport to the atmosphere by contaminated dust particles, direct volatilization from the surface or near surface zones ($\leq$5 cm), and transport to surface water by eroded soil.

Both the calculated and the experimental results show that the PCDDs will concentrate in sediments and biota present in aquatic media. It has been shown by static test procedures that, depending on the species, the bioconcentration factor (BCF) for 2,3,7,8-TCDD in fish ranges from ~2000-30,000. The U.S. EPA's best estimate of the BCF for 2,3,7,8-TCDD is 5000 (U.S. EPA, 1984).

In mammals, 2,3,7,8-TCDD is readily absorbed through the gastrointestinal tract, and absorption through intact skin has also been reported. Absorption may decrease dramatically if 2,3,7,8-TCDD is adsorbed to particulate matter such as activated carbon or soil. After absorption, 2,3,7,8-TCDD is distributed to tissues high in lipid content; however, in many species, the liver is a major storage site. Metabolism of 2,3,7,8-TCDD occurs slowly, with the polar metabolites excreted in the urine and feces. Unmetabolized 2,3,7,8-TCDD can be eliminated in the feces and in the milk. It is metabolized by the P-450 monooxygenase system through a reactive epoxide intermediate. The metabolism of 2,3,7,8-TCDD seems to be a detoxification process resulting in the production of metabolites that are less toxic than the parent compound. Available scientific data supports the contention that the toxic response to 2,3,7,8-TCDD exposure is mediation through cytosolic Ah-receptor site binding.

The PCDDs discussed in this document are among some of the most toxic compounds known, with the lowest $LD_{50}$ level for male guinea pigs, the most sensitive species, being 0.6 μg/kg for 2,3,7,8-TCDD. The other congeners are somewhat less toxic; however, the $LD_{50}$ values are still in the μg/kg range. Although 2,3,7,8-TCDD is highly toxic in all species tested, there are large species differences in sensitivity, with the $LD_{50}$ for hamsters being 1157-5051 μg/kg. The characteristic signs and symptoms of lethal poisoning are severe weight loss and thymic atrophy. Death usually occurs many days after the exposure. In rats, rabbits and mice, 2,3,7,8-TCDD produces an acute liver injury that is not observed in either monkeys, hamsters or guinea pigs. In mice, the immune response is also suppressed. After subchronic or chronic exposure to 2,3,7,8-TCDD in rats or mice, the liver appears to be the most severely affected organ, although systemic hemorrhage, edema and suppressed thymic activity are also observed. The limited data available for the other PCDDs indicate that these chemicals produce the same symptoms as 2,3,7,8-TCDD in a given species; however, the doses required are higher.

Humans have been exposed to herbicides and other chlorinated chemicals containing 2,3,7,8-TCDD as a contaminant. The symptoms of toxicity in many cases are similar to those observed in animals, with exposure leading to altered liver function and lipid metabolism, porphyria cutanea tarda, neurotoxicity and pathologic changes in hematologic parameters. In addition, exposure of humans to 2,3,7,8-TCDD produces skin lesions such as chloracne and hyperpigmentation. Although some signs such as chloracne are attributed to the PCDDs, the other signs of toxicity may arise, at least in part, from the other chemical of which PCDDs are a minor contaminant.

Animal studies have demonstrated that 2,3,7,8-TCDD is teratogenic and fetotoxic in rats, mice, rabbits and ferrets; and fetotoxic in monkeys.

Exposure to 2,3,7,8-TCDD in mice produces facial clefts, while exposure in rats results in edema, hemorrhage and kidney anomalies; rabbits have a higher incidence of extra ribs. In rats a reduction in the gestation index, decreased fetal weight, increased liver-to-body weight ratio and increased incidence of dilated renal pelvis in the offspring has been observed. Certain human epidemiology studies have shown positive associations with exposure to chemicals contaminated with 2,3,7,8-TCDD and birth defects and abortions, while others have not.

There is a limited data base with conflicting evidence for 2,3,7,8-TCDD's mutagenic potential; therefore, the available evidence is judged to be inconclusive. There are no studies in the published literature regarding the mutagenicity of HxCDD or any other congeners of PCDD.

There is evidence from chronic animal cancer bioassay studies that 2,3,7,8-TCDD and HxCDD are probable human carcinogens. There are no chronic cancer bioassay studies available that evaluate the carcinogenic potential for other PCDDs. The available data for 2,3,7,8-TCDD and HxCDD come from gavage and feeding studies, there being no studies available for inhalation exposure. The epidemiologic evidence for the carcinogenicity of 2,3,7,8-TCDD alone is inadequate, while the evidence for phenoxyacetic herbicides and/or chlorophenols with 2,3,7,8-TCDD as an impurity is limited. There have been no epidemiologic evaluations, as yet, for HxCDD as the sole compound of concern.

A number of chronic animal cancer bioassays show that 2,3,7,8-TCDD is an animal carcinogen. In rats, oral exposure to 2,3,7,8-TCDD resulted in an increased incidence of hepatocellular carcinomas, squamous cell carcinomas of the tongue and hard palate/nasal turbinates, and squamous cell carcinomas of the lung. In both male and female mice, increased incidences of liver tumors were observed. A mixture of the two isomers of HxCDD, discussed in this document has been tested for carcinogenicity and shows increased incidences of liver tumors in rats and mice. Also, 2,3,7,8-TCDD has produced fibrosarcomas at the site of application after dermal administration, although there was no significant increase in dermal tumors when the mixture of HxCDDs was tested. Since both compounds produce statistically significant increased incidences of tumors in two species of animals, there is sufficient evidence, according to the interim EPA weight-of-evidence classification criteria, to conclude that both 2,3,7,8-TCDD and HxCDD are animal carcinogens. 2,3,7,8-TCDD has been shown to be a promoter as well as an initiator in rodent test systems. Evidence is available from epidemiologic studies that implicate exposure to herbicides contaminated with 2,3,7,8-TCDD with a significantly elevated risk of soft tissue sarcomas and to a lesser extent non-Hodgkins lymphomas; however, the exposures to 2,3,7,8-TCDD were always compounded with exposures to the herbicide chemicals.

Assuming that 2,3,7,8-TCDD and HxCDD are carcinogenic in humans, upper bound incremental unit cancer risks have been estimated for both ingestion and inhalation exposure. The unit risks have been estimated using a multistage extrapolation model that is linear at low doses. Available metabolism and pharmacokinetic data are insufficient to alter typically used assumptions for estimating the human equivalent dose. Since incidence data exist only for oral studies in animal test systems, the inhalation risk estimates are based upon the cancer potency derived from the oral studies along with appropriate conversion assumptions.

Using data from a feeding study with female rats the upper limit incremental cancer risk for 2,3,7,8-TCDD is estimated to be $1.56\text{x}10^{-1}$ per ng/kg/day. The upper limit estimate of incremental cancer risk is

$4.5\times10^{-3}$ for a continuous lifetime exposure to 1 ng/$\ell$ of 2,3,7,8-TCDD in drinking water and $3.3\times10^{-5}$ for a continuous lifetime exposure to 1 pg/$m^3$ of 2,3,7,8-TCDD in ambient air.

Using data from an ingestion study with female rats and male mice, the cancer potency for HxCDD is estimated to be $6.2\times10^{-3}$ per ng/kg/day. The upper limit estimate of incremental cancer risk is $1.8\times10^{-4}$ for a continuous lifetime exposure to 1 ng/$\ell$ of HxCDD in drinking water and $1.3\times10^{-6}$ for a continuous lifetime exposure to 1 pg/$m^3$ of HxCDD in ambient air.

## 2.2. CONCLUSIONS

The PCDDs, 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 1,2,3,6,7,8- and 1,2,3,7,8,9-HxCDD, are highly toxic following acute exposure. All animal species administered high levels of these compounds developed weight loss and thymic atrophy. In some species liver damage, edema, hair loss and immunosuppression were also observed. Chronic toxicity studies have been conducted only on 2,3,7,8-TCDD and a mixture of the two isomers of HxCDD. In these studies, the primary nonneoplastic lesion was fatty and necrotic change in the liver.

In the species studied, the fetus has been shown to be highly sensitive to the toxic effects of 2,3,7,8-TCDD. In rats the fetotoxicity observed included hemorrhage, edema and kidney anomalies, while in mice the predominant lesions were cleft palate and kidney anomalies. The lowest reported exposure in rats, 1 ng/kg, produced a significant (by some analyses but not others) effect on the fetus, and was similar to the LOAEL observed in chronic studies.

Evidence from oral animal cancer bioassays is "sufficient" (according to EPA and IARC criteria) to conclude that 2,3,7,8-TCDD and a mixture of the two isomers of HxCDD are animal carcinogens. 2,3,7,8-TCDD has increased the incidence of a variety of tumors, including hepatocellular tumors in rats and mice, while the mixture of HxCDD tested increased the incidence of hepatocellular tumors in both sexes of rats and mice. The available epidemiologic evidence for the carcinogenicity of 2,3,7,8-TCDD alone is inadequate and there have been no epidemiologic evaluations, as yet, for HxCDD as the sole compound of concern. Considering the animal evidence together with the epidemiologic data, the overall weight-of-evidence classification for 2,3,7,8-TCDD using EPA's interim classification scheme is category B2 meaning that 2,3,7,8-TCDD should be regarded as a "probable" human carcinogen. The overall weight-of-evidence classification for HxCDD is also category B2 meaning that it should be regarded as a "probable" human carcinogen. In terms of low dose potency, 2,3,7,8-TCDD and the HxCDD mixture are the two most potent carcinogens evaluated by the EPA's Carcinogen Assessment Group. Epidemiologic studies of workers exposed to chemicals contaminated with 2,3,7,8-TCDD such as 2,4,5-trichlorophenoxyacetic acid and 2,4,5-trichlorophenol have produced positive findings that are suggestive of an elevated risk of cancer in humans. These epidemiologic findings are not inconsistent with the premise that 2,3,7,8-TCDD is probably carcinogenic for humans. There are no chronic studies available regarding the carcinogenicity of 1,2,3,7,8-PeCDD.

## 2.3. NEEDS FOR FUTURE RESEARCH

- The basic physical properties such as water solubilities and vapor pressures of the PeCDDs and HxCDDs need to be determined. These parameters are important in predicting the environmental fate of these compounds.
- New analytical methodologies must be established to determine the low levels of these compounds in environmental matrices without ambiguity.
- More monitoring data, particularly in air and aquatic media as well as in vegetables grown near urban incinerators, should be developed by a diversity of research groups.

- Isotopically labeled internal standard compounds ($^{37}$Cl or $^{13}$C) should be prepared for PeCDDs and HxCDDs.

- More research efforts should be directed to determining the environmental fate of the PeCDDs and HxCDDs. The determination of the fate of these chemicals with respect to the possibility of photochemical transformations in different environmental matrices needs special attention.

- Pharmacokinetic studies should be conducted to demonstrate more clearly the degree of absorption of the PCDDs by all routes. In particular, studies are needed on respiratory absorption and on PCDDs adsorbed to environmental media.

- Although a number of studies demonstrate that 2,3,7,8-TCDD is a teratogen, the other congeners should be tested for teratogenic potential.

- There is no information on the effects of chronic exposure to 1,2,3,7,8-PeCDD, and studies should be conducted to determine both the toxic effects of this compound and its carcinogenic potential.

- Further epidemiology data on the effects in human populations exposed to PCDDs might assist in determining which effects observed in animals are also present in humans. In these studies, careful quantitation of PCDD levels in humans and industrial hygiene samples might provide dose-response data necessary for health assessment.

- Bioavailability studies from contaminated soil, fly ash, etc., are needed.

- Mechanism-of-action studies should be conducted to determine the fundamental mode of action of the PCDDs.

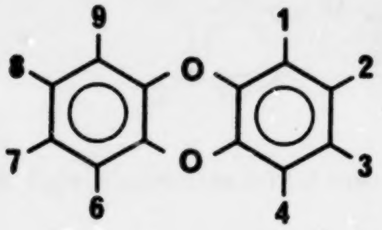
- New destruction methods should be investigated in order to provide feasible methods for decontaminating environmental sites where PCDDs have been detected.

- Determination of BCF for all these most toxic PCDDs in state-of-the-art test systems.

# 3. PHYSICAL AND CHEMICAL PROPERTIES/ANALYTICAL METHODOLOGY

## 3.1. INTRODUCTION

Dibenzo-p-dioxin is a derivative of the basic chemical structure p-dioxane. The structure of dibenzo-p-dioxin and the conventional numbering system used for defining substituent positions are shown below:

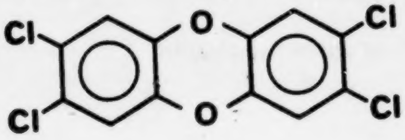


A number of substituents including nitro, amino, alkyl, alkoxy and halogen can be introduced at the different positions of the two benzene rings. Most environmental interest in substituted dibenzo-p-dioxins and most studies of this family of compounds have centered on chlorinated dibenzo-p-dioxins that are loosely referred to as "dioxins." Theoretically, there are 75 different congeners of chlorinated dibenzo-p-dioxins. In this document, only four polychlorinated dibenzo-p-dioxins, namely 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD), 1,2,3,7,8-pentachlorodibenzo-p-dioxin (1,2,3,7,8-PeCDD), 1,2,3,6,7,8-hexachlorodibenzo-p-dioxin (1,2,3,6,7,8-HxCDD) and 1,2,3,7,8,9-hexachlorodibenzo-p-dioxin (1,2,3,7,8,9-HxCDD) will be discussed.

## 3.2. PHYSICAL AND CHEMICAL PROPERTIES

### 3.2.1. Chemical Formula and Synonyms.

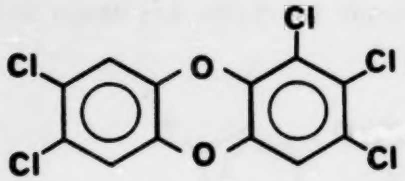
2,3,7,8-Tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD)



Chem. Abstr. Name: 2,3,7,8-tetrachlorodibenzo[b,e](1,4)-dioxin

Synonyms: Dioxin; TCDBD; TCDD; 2,3,7,8-tetrachlorodibenzodioxin, 2,3,7,8-tetrachlorodibenzo-1,4-dioxin.

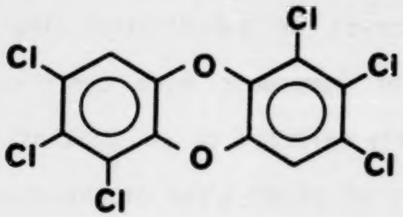
1,2,3,7,8-Pentachlorodibenzo-p-dioxin (1,2,3,7,8-PeCDD)



Chem. Abstr. Name: 1,2,3,7,8-Pentachlorodibenzo[b,e](1,4)dioxin

Synonym: 1,2,3,7,8-Pentachlorodibenzodioxin

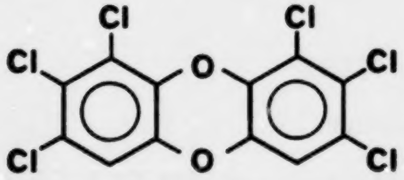
1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin (1,2,3,6,7,8-HxCDD)



Chem. Abstr. Name: 1,2,3,6,7,8-Hexachlorodibenzo[b,e](1,4)dioxin

Synonym: 1,2,3,6,7,8-Hexachlorodibenzodioxin

1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin (1,2,3,7,8,9-HxCDD)



Chem. Abstr. Name: 1,2,3,7,8,9-Hexachlorodibenzo[b,e](1,4)dioxin

Synonym: 1,2,3,7,8,9-Hexachlorodibenzodioxin

3.2.2. Physical Properties. The physical properties of the four polychlorinated dioxins are given in Table 3-1. Although the physical properties of 1,2,3,7,8-PeCDD, 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD have not been well studied, these properties have been more intensively studied for 2,3,7,8-TCDD. 2,3,7,8-TCDD is lipophilic, exhibiting a higher degree of solubility in fats and oils than in water. The solubility of 2,3,7,8-TCDD in various solvents (at unspecified temperatures) is as follows (Crummett and Stehl, 1973):

| Solvent | Solubility (ppm) |
|---|---|
| water | $2 \times 10^{-4}$ |
| lard oil | 44 |
| benzene | 570 |
| o-dichlorobenzene | 1400 |
| chloroform | 370 |
| acetone | 110 |
| n-octanol | 50 |
| methanol | 10 |

The solubilities of HxCDD (isomer unspecified) in benzene and toluene are 1600 and 1800 ppm, respectively (U.S. EPA, 1978). The known solubility data (NRCC, 1981a) suggest that while the lower congeners (e.g., di-CDD and tri-CDD) are more soluble in aliphatic solvents (e.g., acetone, methanol), the higher homologues are more soluble in aromatic hydrocarbon solvents. However, the solubilities of both lower and higher homologues of polychlorinated dioxins may be comparable in chlorinated aliphatic hydrocarbons, namely chloroform.

Because of the $\pi \rightarrow \pi^*$ transitions the polychlorinated dioxins have two absorption maxima in the near UV region. The absorption coefficients resulting from this transition at longer wavelengths are presented in Table 3-1. The partition coefficient of 2,3,7,8-TCDD in a hexane water system was estimated to be 1000 (temperature unspecified) (Matsumura and Benezet,

TABLE 3-1

Physical Properties of a Few Selected Polychlorinated Dioxins

| Compound | CAS Reg. No. | Molecular Formula | Molecular Weight | Description | Melting Point (°C) | $\lambda_{max}$[a] (chloroform) (nm) | $E_{1}^{1}$[b] | Reference |
|---|---|---|---|---|---|---|---|---|
| 2,3,7,8-TCDD | 1746-01-6 | $C_{12}H_4Cl_4O_2$ | 321.9 | colorless needles | 305-306 | 310 | 173.6 | Pohland and Yang, 1972 |
| 1,2,3,7,8-PeCDD | 40321-76-4 | $C_{12}H_3Cl_5O_2$ | 356.5 | NA | 240-241 | 308 | 171.4 | Gray et al., 1976 |
| 1,2,3,6,7,8-HxCDD | 57653-85-7 | $C_{12}H_2Cl_6O_2$ | 390.9 | NA | 285-286 | 316 | 152 | Gray et al., 1975 |
| 1,2,3,7,8,9-HxCDD | 19408-74-3 | $C_{12}H_2Cl_6O_2$ | 390.9 | NA | 243-244 | 317 | 104 | Gray et al., 1975 |

[a] This is the wavelength of maximum absorption.

[b] This is the absorption coefficient for a 1% chloroform solution of substrate in 1 cm cell at the $\lambda_{max}$. To convert this to the molar absorption coefficient ($M^{-1}$ $cm^{-1}$), multiply by one-tenth of the molecular weight.

NA = Not available

1973). Values for other physical properties for these compounds have been estimated from various correlation equations and are given in Table 3-2.

The infrared, mass, phosphorescence, and nuclear magnetic spectra of 2,3,7,8-TCDD are available from various sources (Mahle and Shadoff, 1982; Pohland and Yang, 1972; Chen, 1973; Kende and Wade, 1973). The mass spectra of the three other PCDDs are also available (Mahle and Shadoff, 1982; Gray et al., 1975, 1976). The response ratios of electron impact (EI) and negative chemical ionization (NCI) and fragmentation of 11 of the TCDD isomers have been reported by Rappe et al. (1983a). These spectra, particularly the mass spectra, are very useful in identifying the various homologues/isomers of the PCDDs, but they give limited information for the identification of particular isomers.

3.2.3. Chemical Properties. All four PCDDs are rather stable toward heat, acids and alkalies, although heat treatment with alkali (under conditions similar to alkaline extraction of tissue) completely destroys octa-CDD (Albro, 1979). These compounds begin to decompose at 500°C, and at a temperature of 800°C, virtually complete degradation of 2,3,7,8-TCDD occurs within 21 seconds (Stehl et al., 1973). The PCDDs are susceptible to photodegradation in the presence of UV light. They also undergo photoreductive dechlorination in the presence of an effective hydrogen donor. Gamma radiation degrades 2,3,7,8-TCDD in organic solvents (Fanelli et al., 1978).

## 3.3. ANALYTICAL METHODOLOGY

Several publications on the analytical methods for the determination of PCDD levels in different media are available. The analytical methodologies for the separation of the different isomers of PCDDs are difficult and expensive. Many investigators, particularly the earlier ones, failed to characterize the individual isomers and it is not always clear whether a

## TABLE 3-2

### A Few Estimated Physical Parameters of Chlorinated Dibenzo-p-Dioxins[a]

| Parameter | 2,3,7,8-TCDD | PeCDD[b] | HxCDD[b] |
|---|---|---|---|
| Vapor pressure (mm of Hg) at 25°C and 1 atmosphere | $1.7 \times 10^{-6}$<br>$1 \times 10^{-6}$[c] | NA | NA |
| Octanol/water partition coefficient at 25°C | $1.4 \times 10^{6}$<br>$6.9 \times 10^{6}$[c]<br>$1.9 \times 10^{7}$[d]<br>$1.4 \times 10^{6}$[e] | $7 \times 10^{6}$ | $4.2 \times 10^{7}$ |
| Sorption partition coefficient ($K_{OC}$) | $9.9 \times 10^{5}$<br>$3.3 \times 10^{6}$[c] | $5 \times 10^{6}$ | $3 \times 10^{7}$ |
| Water solubility (ppb) at 25°C | 0.2[f] | 0.04 | 0.008 |

[a] Source: NRCC, 1981a (unless otherwise stated), based on vapor pressure data (Firestone, 1977a) and the octanol/water partition coefficient value (Kenaga, 1980)

[b] These are estimated values for nonspecific isomers

[c] Mabey et al., 1981

[d] U.S. EPA, 1984

[e] This is a measured value (Neely, 1979)

[f] This is the experimental value (Crummett and Stehl, 1973)

NA = Not available

specific isomer or a mixture of isomers was responsible for the observed effect(s). However, analytical methods for detecting specific isomers at low ppt levels are now available for human samples (Crummett, 1983). In the case of TCDDs, the specific isomer 2,3,7,8-TCDD has been more thoroughly studied than any of its other isomers because of its high toxicity. It is not the purpose of this section to review the various analytical methodologies available for PCDDs. Such reviews of recent analytical methods have been done in a Canadian document (NRCC, 1981b), a U.S. EPA (1980a) report and by Tiernan (1983). Instead, this section will attempt to point out the various problems that may be encountered in the analysis of these compounds and provide a critique of a few typical analytical methods available for PCDDs.

3.3.1. General Procedure for the Analysis of PCDDs. The analysis of PCDDs can be broadly divided into three basic steps (sample preparation, sample cleanup and sample analysis). The description of each of these steps with the associated difficulties that may be encountered are discussed below.

3.3.1.1. SAMPLE PREPARATION -- In this step, the sample is homogenized or digested and extracted with a suitable solvent or a solvent mixture to remove the bulk of the sample matrix and to transfer the PCDD residue into the solvent(s). Both the selection of the proper solvent(s) and the method of extraction can be critical in obtaining a satisfactory recovery of PCDDs from the sample matrix. A number of solvents including hexane, hexane-acetone, benzene, toluene, chloroform and methylene chloride generally have been used for extracting PCDDs from sample matrix (Kooke et al., 1981; Harless et al., 1980; Van Ness et al., 1980). If the sample does not contain water, as is the case with fly ash and atmospheric particulate samples, either benzene or toluene appears to be the desirable solvent

(Kooke et al., 1981). Toluene should be preferred over benzene, however, because of its lower toxicity. For the extraction of PCDDs from aquatic media, a solvent leading to high partition coefficient should be selected. No systematic study, however, has been done on the extractability of these compounds from aquatic media by different solvents.

The lipid content of different tissues may also influence the amount and the nature of extraction solvent. For example, chloroform-methanol is effective for serum and plasma, but it produces emulsion with milk containing higher lipid (Albro, 1979).

In other sample matrices that contain high amounts of water, such as tissues and food samples, the water may alter the extractability of a solvent. For example, although acetone may be a good solvent for soil extraction, the admixture of a small amount of water decreases the solubility of the substrate so that it cannot be used directly for animal tissues. Mixtures of polar and nonpolar solvents such as benzene-methanol may separate into two phases in the presence of 2% water, resulting in nonreproducible extraction (Albro, 1979).

Samples that may contain PCDDs bound to the matrices, such as tissue, food, soil and sediment, may require acid/base digestion procedures to release the bound substrate into the extraction media. The acid/base extraction is normally done with concentrated acid or an alcoholic base (Tosine, 1981; Harless et al., 1980). Kooke et al. (1981) reported highest extraction efficiencies by acid treatment of fly ash before extraction. The increase in efficiency was hypothesized to be due to opening of some of the pores in the fly ash structure, thus making the solvent more accessible to the sorbed PCDDs. Refluxing with alkaline potassium hydroxide, however, may cause decomposition of the higher polychlorinated dioxins and oxidation of some products (Hass and Friesen, 1979; Albro, 1979). A neutral extraction system is reported to circumvent the possibility of this loss and has been used by several authors (O'Keefe et al., 1978; Harless et al., 1980).

The extraction efficiency may also depend on the method of extraction. The extraction efficiencies of PCDDs by simple shaking, ultrasonication and soxhlet extraction were studied by a few investigators (Kooke et al., 1981; Chess and Gross, 1980). While Chess and Gross (1980) reported no significant improvement in extraction efficiencies of PCDDs from fly ash by sonication or soxhlet extraction, Kooke et al. (1981) found soxhlet extraction to be a better procedure than the other two methods. Similarly, Albro (1979) reported that the nature of the sample matrix influences the effectiveness of extraction. Thus, while it may be possible to extract liver in a Teflon-glass homogenizer, brain tissues may require a blender, and skin a powerful disintegrator such as the Polytron for the extraction of residues.

3.3.1.2. SAMPLE CLEANUP -- The sample cleanup procedure normally consists of three essential steps. A fourth step is usually required if an isomer specific identification and quantification is required. The first step in the cleanup procedure consists of the removal of lipids from the extracted sample matrix. The lipid cleanup can be achieved by two routes, namely, solvent extraction or reaction with an acid or a base. The use of solvents such as hexane, hexane-acetone, chloroform, chloroform-methanol and petroleum ether (NRCC, 1981b) is common. The use of nonpolar solvents (hexane or $CCl_4$) gives excellent results when lipids consist primarily of triglycerides and/or phospholipids. When the lipid consists of cholesterol esters, however, sulfuric acid treatment gives a better result than nonpolar solvent extraction (Albro, 1979). Similarly, base wash of the organic phase may remove interfering lipids and other materials through saponification, hydrolysis or degradation. However, acid wash is more commonly used than base wash presumably because of the probability of decomposition (Albro, 1979) and oxidation (Hass and Friesen, 1979) of sample components as a result of base wash. The possibility of decomposition of higher PCDDs by the base may be the reason for its less frequent use. It should be mentioned that some investigators used chromatographic columns such as silica gel containing sulfuric acid for the acid/base cleanup step instead of washing off the lipids by simple shaking (Lamparski et al., 1979; Fanelli et al., 1980a; Langhorst and Shadoff, 1980; Buser, 1978; DiDomenico et al., 1980a).

The second step in the cleanup procedure consists of removal of common impurities such as pesticide residues from the PCDDs. Liquid chromatography with alumina, Florisil, silica, foam charcoal or carbon dispersed on glass fibers has been used for this purpose (Harless et al., 1980; Mitchum et al., 1980; Chess and Gross, 1980; Buser, 1978; Tiernan et al., 1980; Stalling et al., 1983; Buser and Rappe, 1983). A few investigators have used $AgNO_3$-impregnated silica gel columns (Lamparski et al., 1979; Tosine, 1981; Langhorst and Shadoff, 1980). The $AgNO_3$/silica column system is claimed to be effective in the removal of DDE, chlorinated aliphatics and sulfides.

There is a difference between the various alumina columns (Lamparski et al., 1979; Harless et al., 1980). The separation of PCDDs from PCBs may be accomplished with acidic, neutral and basic alumina; most authors have provided no reason for choosing one over the other. However, it has been shown by Albro (1979) that acidic alumina may be better than basic alumina, which in turn may be better than neutral alumina for the separation of residual lipids from the PCDDs in the sample extracts.

The third step in the cleanup procedure is used solely as an additional cleanup of contaminants and has been used by a few investigators (Langhorst and Shadoff, 1980; Lamparski et al., 1979; Mitchum et al., 1980). The removal of these additional impurities has been obtained by using HPLC with both normal and reversed phase packing materials. Recently, Phillipson and Puma (1980) reported that chlorinated methoxybiphenyls in fish extract could coelute with TCDDs through an alumina-Florisil cleanup sequence and interfere with the determination of TCDDs. A few compounds that may interfere with the determination of TCDD at m/e values of 319.8966 and 321.8936 are given in Table 3-3.

The additional cleanup step using the HPLC separation procedure may be essential for the unequivocal separation of impurities that may interfere with the MS analysis of PCDDs.

The fourth and final cleanup step consists of the separation of PCDDs into several different fractions by means of chromatographic techniques. Both liquid chromatography with alumina columns (Hass et al., 1978; Albro and Corbett, 1977) and HPLC with normal and reverse phases have been used (Tosine, 1981; Ryan and Pilon, 1980; Langhorst and Shadoff, 1980; Mitchum et al., 1980). The separation of PCDDs using HRGC is necessary for the unequivocal separation of 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD and 1,2,3,7,8,9-HxCDD from the other congeners. Buser and Rappe (1983) have shown that this separation can be achieved using a 55 m Silar column. The unequivocal separation of 2,3,7,8-TCDD from other isomers has been accomplished by a combination of reverse phase and normal phase HPLC, and packed column GLC by Langhorst and Shadoff (1980). The cleanup procedure used by most of the other investigators has failed to demonstrate this unequivocal separation of all the TCDD isomers. The various cleanup and analysis procedures have been compared by Brumley et al. (1981).

TABLE 3-3

Potential Interferences in the Determination of TCDDs at m/e Values of 319.8966 and 321.8936*

| Compound | Molecular Formula | Interfering Ion | m/e | Resolution for Separation |
|---|---|---|---|---|
| Heptachlorobiphenyls | $C_{12}H_3{}^{35}Cl_7$ | $M^+ -2{}^{35}Cl$ | 321.8678 | 12476 |
| Nonachlorobiphenyls | $C_{12}H\ {}^{35}Cl_9$<br>$C_{12}H\ {}^{35}Cl_8\ {}^{37}Cl$ | $M^+ -4{}^{35}Cl$<br>$M^+ -3{}^{35}Cl\ {}^{37}Cl$ | 319.8521<br>321.8491 | 7189<br>7233 |
| Tetrachloromethoxy biphenyls | $C_{13}H_8\ {}^{35}Cl_4O$<br>$C_{13}H_8\ {}^{35}Cl_3\ {}^{37}ClO$ | $M^+$<br>$M^+$ | 319.9329<br>321.9299 | 8805<br>8848 |
| Tetrachlorobenzyl-phenyl ethers | $C_{13}H_8\ {}^{35}Cl_4O$<br>$C_{13}H_8\ {}^{35}Cl_3\ {}^{37}ClO$ | $M^+$<br>$M^+$ | 319.9329<br>321.9300 | 8813<br>8843 |
| Pentachlorobenzyl-phenyl ethers | $C_{13}H_7\ {}^{35}Cl_4\ {}^{37}ClO$<br>$C_{13}H_7\ {}^{35}Cl_3\ {}^{37}Cl_2O$ | $M^+ -H{}^{35}Cl$<br>$M^+ -H{}^{35}Cl$ | 319.9143<br>321.91138 | 18043<br>18104 |
| DDT (4 isomers) | $C_{14}H_9\ {}^{35}Cl_3\ {}^{37}Cl_2$<br>$C_{14}H_9\ {}^{35}Cl_2\ {}^{37}Cl_3$ | $M^+ -H{}^{35}Cl$<br>$M^+ -H{}^{35}Cl$ | 319.9321<br>321.92917 | 9006<br>9050 |
| DDE (4 isomers) | $C_{14}H_8\ {}^{35}Cl_2\ {}^{37}Cl_2$<br>$C_{14}H_8\ {}^{35}Cl\ {}^{37}Cl_3$ | $M^+$<br>$M^+$ | 319.9321<br>321.92916 | 9011<br>9052 |
| Hydroxytetrachloro-dibenzofurans | $C_{12}H_4Cl_4O_2$ | $M^+$ | 319.8966<br>321.8936 | NR<br>NR |
| Tetrachlorophenyl-benzoquinones | $C_{12}H_4Cl_4O_2$ | $M^+$ | 319.8966<br>321.8936 | NR<br>NR |
| Tetrachloroxanthenes | $C_{13}H_6O\ {}^{35}Cl_3\ {}^{37}Cl$<br>$C_{13}H_6O\ {}^{35}Cl_2\ {}^{37}Cl_2$ | $M^+$<br>$M^+$ | 319.9143<br>321.9114 | 18043<br>18104 |

*Source: NRCC, 1981b

NR = Not resolved by MS

The cleanup of the samples through liquid chromatography with subsequent quantification of PCDDs requires concentration of the sample solution. Evaporation to dryness by an inert gas stream appears to be an accepted procedure for concentrating the TCDD solutions. If the concentration procedure is not properly controlled, it can introduce error in two different ways. It has been shown by Lamparski et al. (1979) that concentration of sample solution with prepurified nitrogen can introduce severe contamination. Therefore, further purification of the gas stream with a series of traps containing 10% Apiezon L plus 10% each micronized Carbopack B and Amoco PX-21 on 60/80 Chromosorb W-AW, 13 x molecular sieve, 20% $H_2SO_4$ on Bio-Sil A, and Carbosieve 8S were required. Secondly, O'Keefe et al. (1982) have demonstrated that significant losses of 2,3,7,8-TCDD occur when nitrogen evaporation to dryness is done at temperatures >50°C.

3.3.1.3. **SAMPLE ANALYSIS** -- The final analysis of PCDDs is almost exclusively performed by GC/MS. Although some of the earlier investigators (Lamparski et al., 1978; Firestone, 1977b) used GC with electron capture detection, it does not have the sensitivity for complex samples containing low levels ($<10$ ng $kg^{-1}$) of PCDDs (Hass and Friesen, 1979).

The final separation procedure for PCDD analysis uses GC with packed or capillary columns. A typical list of packed and capillary columns used for the analysis of PCDDs is given in Table 3-4. Capillary columns are preferable over packed columns because they provide better separation of components in a complex mixture than packed columns. There are other advantages of capillary columns, namely, that the narrow band width of the separated components enhances MS sensitivity, and the capillary columns with their low bleed rates enhance MS sensitivity by keeping the background contamination low. A disadvantage of the capillary columns relative to the packed columns

## TABLE 3-4

## Some Packed and Capillary Columns Used for the Analysis of PCDDs

| | |
|---|---|
| **PACKED COLUMNS** | |
| 1.8 m x 2 mm i.d., 3% Dexsil 300 | Van Ness et al., 1980 |
| 0.6-2 m x 2.5 mm i.d., 3% OV-1, 3% OV-17, 3% OV-61, 2% OV-101 | DiDomenico et al., 1980a |
| 1.8 m x 2 mm i.d., 3% OV-7 | Tiernan et al., 1980 |
| 2 m x 2 mm i.d., 3% OV-210 | Parker et al., 1980 |
| 2 m x 2 mm i.d. specially packed 0.2% carbon wax 20 M (Aue packing) | Eiceman et al., 1981 |
| 2 m x 2 mm i.d., 0.6% OV-17/0.4% Poly S179 | Langhorst and Shadoff, 1980 |
| 2 m x 4 mm i.d., 1.2% Silar 10C | Firestone et al., 1979 |
| 1.8 m x 2 mm i.d., 5% SE-30 | Baughman and Meselson, 1973 |
| **CAPILLARY COLUMNS** | |
| 18 m x 0.3 mm i.d., OV-61 WCOT | Buser, 1975 |
| 22 m x 0.3 mm i.d., OV-17, 101, Silar 10C | Buser, 1976 |
| 50 m x 0.36 mm i.d., OV-17 WCOT | Buser and Rappe, 1978 |
| 30 m x (i.d. not given), SE-30 WCOT | Harless and Oswald, 1978 |
| 30 m x 0.25 mm i.d., OV-101 WCOT | Harless and Lewis, 1980a |
| 30 m x 0.25 mm i.d., SE-30 WCOT | Harless et al., 1980 |
| 20 m, SP-2100 SCOT | Mitchum et al., 1980 |
| 25 m x 0.2 mm i.d., quartz, methyl silicone WCOT | Norstrom et al., 1982 |
| 30 m x 0.5 mm i.d., glass, 60/40 w/w OV-17/ Poly S-179 | Nestrick et al., 1980 |
| 50 m x 0.25 mm i.d., glass Silar 10C | Buser and Rappe, 1980 |
| 55 m x 0.37 mm i.d., glas OV-17 | Buser and Rappe, 1980 |
| 55 m x 0.40 mm i.d., glass OV-101 | Buser and Rappe, 1980 |
| 60 m x 0.26 mm i.d., Supelco SP-2330 | Rappe et al., 1983b |
| 50 m x 0.4 mm i.d., OV-101 fused silica | Tiernan, 1983 |
| 60 m OV-101 WCOT (i.d. unspecified) | Van Ness et al., 1980 |

is the problem of easy overload in the presence of other coextracted impurities. One group of researchers (Langhorst and Shadoff, 1980) has used a packed column for the unequivocal determination of 2,3,7,8-TCDD in the presence of 21 other isomers. However, this determination was possible because of the prior separation of components through fractionation by HPLC with a combination of a reverse phase Zorbax ODS column and a normal phase silica column. DiDomenico et al. (1980a) also found low resolution GC suitable for the analysis of ppt levels of TCDDs in environmental samples, provided the samples are adequately precleaned. Although the analysis of environmental samples from the Seveso accident by DiDomenico et al. (1980a) may not have required HRGC column because no other isomers were expected to have been formed (Buser, 1978), a packed column may not be satisfactory for the unequivocal determination of 2,3,7,8-TCDD in the presence of interference from other TCDD isomers (Hummel and Shadoff, 1980).

The separation of 2,3,7,8-TCDD from all the other 21 isomers is difficult even with capillary columns. A combination of OV-101 and OV-17 glass capillary columns of 20-30 m length and 0.35-0.37 mm i.d. was required for unequivocal separation of 2,3,7,8-TCDD from the other 21 isomers of TCDD (Buser, 1978). However, a Silar 10C glass capillary column of 55 m length and 0.25 mm i.d., and with a theoretical plate number of 192,000, provided almost unambiguous separation of 2,3,7,8-TCDD from its other isomers (Buser and Rappe, 1980). Other capillary columns known to separate 2,3,7,8-TCDD from the other TCDD isomers include SP-2340, SP-2330 and Silov (Tiernan, 1983). A 50 m length of a Silar 10C capillary column has been recommended by the U.S. EPA (1982a) for the determination of 2,3,7,8-TCDD in municipal and industrial wastewaters. The same column can also be used for the unequivocal separation of 1,2,3,7,8-PeCDD and 1,2,3,6,7,8- and 1,2,3,7,8,9-HxCDD from the less toxic congeners.

As previously mentioned, MS is used almost exclusively for the detection and quantification of PCDDs. Basically, three MS techniques (LRMS, HRMS and NICI) have been used. A few different MS systems used for the determination of TCDDs are shown in Table 3-5. It is obvious from Table 3-5 that electron impact ionization in the low resolution mode (resolution <8000, 10% valley) has been the most widely applied MS method used for the determination of TCDDs.

The electron-impact mass spectra of PCDDs show strong molecular ions ($M^+$). Fragmentation occurs through the loss of CO and Cl radicals. Major ions are at $M^+$-63 ($M^+$-COCl) and $M^+$-126 ($M^+$-2COCl). Doubly charged molecular ions ($M^{2+}$) and minor fragmentation ions occur at $M^+$-35 ($M^+$-Cl), $M^+$-70 ($M^+$-2Cl) and $M^+$-98 ($M^+$-COCl-Cl). The usual characteristic ion clusterings caused by the chlorine isotopes are also observed. Based on molecular ions and fragmentation pattern, PCDDs can be distinguished from other chlorinated pollutants. However, this requires monitoring multiple ions. The ions that are commonly monitored for 2,3,7,8-TCDD are $M^+$ and its chlorine isotope clusters, that is, 320 ($^{35}Cl_4$ CDD), 322 ($^{35}Cl_3{}^{37}Cl$ CDD) and 324 ($^{35}Cl_2{}^{37}Cl_2$ CDD). In some instances, fragment ions at 257 (320-CO$^{35}$Cl), 259 (322-CO$^{35}$Cl) and 194 (320-2CO$^{35}$Cl) are also monitored. The intensity ratios in the mass spectrometric peaks that are due to chlorine isotope proportions in native TCDD can be used for assessing the degree of interference and confirming the identity of the TCDDs. Thus, the relative peak intensities of pure 2,3,7,8-TCDD at 320:322:324 are expected to be 77:100:49 (NRCC, 1981a). The response for the ion at 257 is ~30% of the response for the ion at 322 (Glaser et al., 1981). Sometimes internal standards containing ($C_{12}H_4{}^{37}Cl_4O_2$) or ($^{13}C_{12}H_4{}^{35}Cl_4O_2$) used for TCDD analysis give prominent ion peaks at 328 and 332, respectively. The primary

## TABLE 3-5

The Detection Limit, Resolution and Ions Monitored by a Few Mass Spectrometric Systems for the Determination of TCDDs[a]

| Ionization Method and Reference | TCDD Limit of Detection (pg) | M/ΔM[b] | m/e Values Monitored for TCDD | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 320 | 322 | 324 | 326 | 328 | 332 | 259 | 257 | 194 |
| ELECTRON IMPACT | | | | | | | | | | | |
| Baughman and Meselson, 1973 | 5 | 10,000 | + | + | | | + | | | + | |
| Crummett and Stehl, 1973 | 6 | 600 | + | + | + | | | | | | |
| Hummel, 1977 | 5-10 | 400 | + | + | | | + | | | | |
| Hummel, 1977 | 5-10 | 3,000 | + | + | | | + | | | | |
| Mahle et al., 1977 | 5 | NR | + | + | | | + | | | | |
| Adamoli et al., 1978 | 50 | unit | + | + | + | | | | | | |
| Adamoli et al., 1978 | 50 | unit | + | + | + | | | | | | |
| O'Keefe et al., 1978 | NR | 10,000 | + | + | | | + | | | | |
| DiDomenico et al., 1980a | 20 | unit | + | + | + | | | | | | |
| Buser and Rappe, 1980 | | unit | + | + | + | | | | | | |
| Cavallaro et al., 1980a | 40-80 | unit | + | + | + | | + | | | | |
| Chess and Gross, 1980 | 50 | 2,000 | + | + | | | | | + | + | |
| Fanelli et al., 1980a | 250 | 400 | + | + | | | | | | | |
| Harless et al., 1980 | 5-10 | 9,000 | + | + | | + | + | | + | + | |
| Langhorst and Shadoff, 1980 | 5 | 1,000 | + | + | | | | + | | | |
| Lamparski and Nestrick, 1980 | 40-60 | unit | + | + | + | | | + | | | |

TABLE 3-5 (cont.)

| Ionization Method and Reference | TCDD Limit of Detection (pg) | M/ΔM[b] | m/e Values Monitored for TCDD | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 320 | 322 | 324 | 326 | 328 | 332 | 259 | 257 | 194 |
| Norstrom et al., 1982 | 5-10[c] | unit | + | + | + | | | | + | | + |
| Tosine, 1981 | 10[c] | unit | + | + | + | | | + | | | |
| Ryan and Pilon, 1980 | 10[c] | 1,000 | | + | | | | | | | |
| Tiernan et al., 1980 | 1[d] | 350 | + | + | | | | | | | |
| Tiernan et al., 1980 | 100[c] | 12,500 | + | + | | + | + | | | | |
| CHEMICAL IONIZATION | | | | | | | | | | | |
| Hass et al., 1978 | 50-500 | unit | 323 for MNCI[e], 252 and 276 for MONCI[f], 176 for ONCI[g] | | | | | | | | |
| Mitchum et al., 1980 | 10 | NR | -176 from 320, -182 from 332 by ONIAPCI[h] | | | | | | | | |

[a] Source: NRCC, 1981a

[b] resolution of mass

[c] ng.kg⁻¹

[d] µg.kg⁻¹

[e] methane negative chemical ionization

[f] methane-oxygen negative ion chemical ionization

[g] oxygen negative ion chemical ionization

[h] oxygen negative in atmospheric pressure chemical ionization

NR = Not reported

$M^+$ ions for PeCDDs and HxCDDs are 356 and 390. If exact masses are used, the normal ion masses at 320, 322, 328, 257 and 259 will correspond to 319.8965, 321.8936, 327.8847, 256.9327 and 258.9298, respectively. Thus, HRMS with appropriate resolution in most cases may positively identify 2,3,7,8-TCDD when the sample cleanup is not specific (Hummel and Shadoff, 1980). However, an unequivocal identification and quantification of 2,3,7,8-TCDD in the presence of its isomers will still require HPLC fractionation or HRGC separation as described earlier.

The following criteria have been outlined by Harless et al. (1980) for confirmation of 2,3,7,8-TCDD residues:

1. Correct GC retention time for 2,3,7,8-TCDD.
2. Correct isotope ratio for the molecular ions 320 and 322.
3. Correct simultaneous response for the molecular ions 320, 322 and 328.
4. Correct responses for the co-injection of sample fortified with $^{37}$Cl-TCDD and 2,3,7,8-TCDD standard.
5. Intensity of molecular ions 320 and 322 must be >2.5 times the noise level.

Supplemental criteria that Harless et al. (1980) suggested for highly contaminated extracts are:

1. COCl loss indicative of TCDD structure.
2. GC/MS peak-matching analysis of molecular ions 320 and 322 in real time to confirm the 2,3,7,8-TCDD elemental composition.

Although the limit of detection for TCDD is about the same on both HRMS and LRMS (Crummett, 1983), the advantage of HRMS over LRMS for PCDD analysis is that the former technique requires far less time-consuming cleanup steps than those required for LRMS although this is dependent on the nature of the sample. With the use of properly selected analytical techniques, the PCDDs can be determined down to sub ppt levels (Crummett, 1983).

The use of chemical ionization techniques has received limited application for the individual TCDD isomers. Other methods not requiring coupling GC with MS have also been used for PCDDs. For example, the method of direct probe and specific ion monitoring ($M^+ \rightarrow M_1^+ + COCl$) based on the concept of MS-MS was used for the analysis of TCDD (Chess and Gross, 1980). Although the method had comparable specificity to GC-HRMS, the precision of the method was not as good.

3.3.2. **Analysis of PCDDs in Specific Environmental Media.** Although the general procedure for the analysis of PCDDs levels has been discussed in Section 3.3.1., the detailed analytical procedures depend on the type of medium. For this document, the environmental media have been divided into four classes, namely, water, air, soil and biological media, and the techniques used for the sampling and analysis of PCDDs in each medium have been discussed individually.

### 3.3.2.1. WATER --

3.3.2.1.1. **Sampling Method** -- Two types of sampling methods can be used for collecting aqueous samples for PCDDs. In the first method, no preconcentration of the samples during collection is made. Grab samples are collected in clean (detergent washed, rinsed with acetone or methylene chloride, and dried) amber glass bottles of 1 ℓ or 1 quart capacity fitted with screw caps lined with Teflon or aluminum foil (U.S. EPA, 1982a). If aluminum foil is used as a liner, it should be washed with acetone and the dull side should face the sample to avoid sample contamination (Albro, 1979). Automatic samplers can also be used for collecting flow proportional composite samples in amber glass bottles (U.S. EPA, 1982a). The sample containers must be kept refrigerated at 4°C and protected from light during compositing. The grab or the composite-samples should be protected from light and be kept at 4°C during shipment. All samples must be extracted within 7 days and completely analyzed within 40 days of extraction (U.S. EPA, 1982a).

The preconcentrative method of sample collection was used by DiDomenico et al. (1980a). In this method, 2-20 ℓ of water was allowed to pass through a 12 cm x 1.5 cm i.d. XAD-2 column at a rate of 60 mℓ/minute. The XAD-2 columns containing the PCDDs should be protected from light and kept at 4°C during transportation and storage.

3.3.2.1.2. Analysis -- Most of the methods found in the literature described 2,3,7,8-TCDD analysis instead of other PCDD analyses in aqueous samples. The methods used for the analysis of 2,3,7,8-TCDD can be used also for the analysis of the other PCDDs. However, the recovery of the individual PCDDs should be established with added internal standards.

An appropriate volume of water (depending on the desired detection limit) with added internal standard of either $^{13}C_{12}$ or $^{37}Cl_4$ 2,3,7,8-TCDD in the amount of 2.5-25 ng (Harless et al., 1980; U.S. EPA, 1982a) can be extracted with hexane (DiDomenico et al., 1980a), methylene chlorine (U.S. EPA, 1982a; Harless et al., 1980) or petroleum ether (Van Ness et al., 1980). Judging from the recovery data (U.S. EPA, 1982a; DiDomenico et al., 1980a; Harless et al., 1980) methylene chloride appears to be a better solvent.

The extract containing 2,3,7,8-TCDD was cleaned by acid and base wash (Harless et al., 1980; U.S. EPA, 1980b; Van Ness et al., 1980) and further cleaned by liquid chromatography with alumina column (Harless et al., 1980; Van Ness et al., 1980). However, U.S. EPA (1982a) recommends another cleanup step using silica gel liquid chromatography, which may be necessary for wastewater but may be unnecessary for drinking water and clean surface water samples. The final separation and analysis was performed by low resolution GC-HRMS (Van Ness et al., 1980; Harless et al., 1980) or high resolution GC-HRMS or LRMS (U.S. EPA, 1982a). If an unequivocal identification of 2,3,7,8-TCDD is required, the U.S. EPA (1982a) method seems to be most appropriate since it recommends using a 50 m Silar 10C capillary column and multiple ion monitoring MS mode that is known to unequivocally identify and quantify 2,3,7,8-TCDD in the presence of its other isomers (Buser and Rappe, 1980). Harless et al. (1980) reported that TCDD in water can be accurately determined to as low a concentration as 0.03 ppt.

3.3.2.2. AIR --

3.3.2.2.1. Sampling Method -- Monitoring of PCDDs from point sources of emission and ambient atmospheric level requires development of sample collection methods from both sources. The available published work suggests that the PCDDs are associated primarily with particulate matters (NRCC, 1981b).

For the collection of air samples from hot point sources, namely exhaust from an incinerator, a number of commercially available sampling probe and sampling trains are available. Most incorporate filters to isolate the particles and a subsequent device to trap gaseous organics from the exhaust. For PCDDs, glass fiber filters of proper pore size are generally used (NRCC, 1981b). The filter should be maintained at a temperature of >100°C to prevent condensation of water. PCDDs that may escape the glass filters may be collected in a polyurethane foam or XAD-2 trap maintained at room temperature. The sampling must be performed in an isokinetic manner to ensure representative sampling. To permit evaluation, the efficiency of the collection method must be documented. The sampling methodology for point sources is in a developmental stage (NRCC, 1981b) and more work is needed in this area. The recommendations for sample collection procedure given above follow the general U.S. EPA procedure for collection of air samples from hot point sources. A modified U.S. EPA Method 5 sampling train (Federal Register, 1971) consisting of a filtering unit, a condenser unit, a resin cartridge unit and a series of impingers have been used by Stanley et al. (1982) to collect PCDDs in flue gas samples from utility boilers.

The collection of PCDDs in ambient atmospheric samples has been achieved by both dustfall jars and high volume samplers (DiDomenico et al., 1980b). Dustfall jars were constructed from 10 ℓ glass vessels topped with metal gridded funnels with a collecting cross section of about 0.11 m². The top of the funnels were about the human breathing level from the ground. The grid allowed particles <500 μm to be collected. Samples were collected for 1 month or the time required for the vessel to be filled with meteroic water and dust. At the end of the sampling time, the liquid phase was separated from the particles by filtration and the two phases were analyzed separately.

The high volume sampling was performed with high volume samplers equipped with A and E glass fiber filters at a flow rate of 1.5 m³/minute (DiDomenico et al., 1980b). The sampling duration was about 160 hours. The whole sampling unit was assembled into a protective container. The efficiency of sample collection by either of the above methods was not established. The high volume sampling can lead to stripping of PCDDs from the filter. A backup filter consisting of polyurethane foam plug may be used to prevent this anticipated loss. Particulate and vapor phase TCDD was also collected by polyurethane foam filters (U.S. EPA, 1982b; Nash and Beall, 1980). The collection efficiency with this system was determined to be 86% by Nash and Beall (1980).

3.3.2.2.2. Analysis -- The analysis of PCDDs in the particulate matter begins with an extraction process. As has been shown in Section 3.3.1.1., the best extraction efficiency is obtained with dilute HCl pretreated particles, followed by soxhlet extraction with benzene or toluene. Liberti and Brocco (1981) found that xylene was a better solvent than toluene, while Cutie (1981) found that o-dichlorobenzene may be better than any of the other solvents. Various extraction procedures for combustion effluent samples have been described by Taylor et al. (1983).

Several methods are available for sample cleanup before analysis. [Basically, the methods used for the analysis of fly ash can be used for particulate matter (Liberti and Brocco, 1981; Eiceman et al., 1980; Tiernan, 1983; Buser et al., 1978)]. In one analytical procedure, Lamparski and Nestrick (1980) added internal standards of $^{13}$C-2,3,7,8-TCDD, $^{13}$C-1,2,3,4,7,8-HxCDD and $^{13}$C-OCDD to the particulate extract. The extract was cleaned with acid and base washes. Next, the extract was cleaned by liquid chromatography with $AgNO_3$/silica column and basic alumina column, followed by cleanup and sample fractionation with an RP-HPLC (Zorbax ODS) and a normal phase HPLC (silica) method. The final analysis was performed with low resolution GC-LRMS. This method provided an unequivocal identification of isomers and permitted analysis of a minimum concentration of 110 ppt of 2,3,7,8-TCDD in electrostatically precipitated fly ash from a municipal burner.

In another method (Rappe et al., 1983b; Buser and Rappe, 1983), the sample (soot or Kleenex tissue from wipe tests) was spiked with 1-5 ng of 2,3,7,8-$^{13}C_{12}$-TCDD, 2,3,7,8-$^{37}Cl_4$-TCDF (tetrachlorodibenzofuran) and $^{37}Cl_8$-OCDD and treated with 1 M hydrochloric acid. The PCDDs and PCDFs in the washed and dried sample were extracted with toluene in a soxhlet extractor and the extract was subjected to column chromatography on silica gel and basic alumina column. The methylene chloride-n-hexane (1:1) fraction from the second column containing PCDDs and PCDFs was subjected to HRGC/MS analysis. A 55 m x 0.26 mm i.d. Silar column was found to be suitable for the isomeric separation of all 22 isomers of TCDD.

### 3.3.2.3. SOIL --

3.3.2.3.1. **Sampling Method** -- Since similar analytical methods are used for both soil and sediments, this subsection describes the sampling and analytical methods for these two sample types.

Whenever possible, the sites for soil samples should be chosen in open areas away from physical obstacles. If the soil is suspected to be contaminated because of fallout from a point source, sampling sites should be established in a grid over a topographical map of the suspected area. Soil samples may be collected by inserting a 0.5 m long and 7 cm i.d. steel cylinder into the soil to a depth of 7 cm and then retracting the soil and the cylinder system. The earth core should be removed and stored in sealed plastic bags (DiDomenico et al., 1980c). The bags should be cooled to 4°C during transportation.

To determine the distribution of PCDDs in soil, samples can be taken from the vertical faces of dug trenches of a maximum depth of 2 m. Suitable steel core cylinders can be inserted horizontally into the trench face from bottom to top. The individual samples collected in this fashion should be stored in plastic bags at 4°C during transportation them. The details of the soil sampling procedure have been described by DiDomenico et al. (1980a,c).

Although no sampling procedure for the collection of sediment samples for PCDD analysis is available, the accepted method (U.S. EPA, 1979a) for the collection of bottom sediments should be adequate in this case. Clam-type or similar dredge samplers, such as Peterson, Shipek or Hopper samplers, can be used to collect sediment sample. Core samplers can also be used for collecting bottom sediments. The collected samples should be stored in glass containers with teflon-lined screw caps, and stored at 4°C during transportation.

3.3.2.3.2. Analysis -- Several methods are available for the analysis of PCDDs in soil samples (Chess and Gross, 1980; Van Ness et al., 1980; Buser, 1978; Buser and Rappe, 1980; Harless et al., 1980). Although most of these methods have been used for the analysis of 2,3,7,8-TCDD, they are applicable for other PCDDs. The methods used for the analysis of soil can also be used with very little modifications for the analysis of sediments.

The first step in the analysis is the extraction of PCDDs from the soil with a suitable solvent or a solvent mixture. A number of solvents including hexane-acetone (1:1), methylene chloride (Buser and Rappe, 1980), aqueous KOH/ethanol (Harless et al., 1980), benzene (Chess and Gross, 1980), petroleum ether (Van Ness et al., 1980), and a number of extraction methods including simple shaking (Van Ness et al., 1980; Buser and Rappe, 1980), refluxing (Harless et al., 1980), sonication and soxhlet extraction (Chess and Gross, 1980), have been used. However, Chess and Gross (1980) demonstrated that, in soil, the results obtained by simple stirring with 1:1 hexane/acetone and the more extensive sonication or soxhlet extraction with benzene are consistent.

The cleanup procedure for the extract generally consists of an acid and base wash, liquid chromatography on silica and alumina columns or two alumina columns, and final analysis by HRGC-LRMS or HRGC-HRMS (Harless et al., 1980; Buser and Rappe, 1980). If an unequivocal identification and quantification of 2,3,7,8-TCDD is required, the 55 m Silar 10C capillary column used by Buser and Rappe (1980) or the 60 m SP-2330 fused silica column (Rappe et al., 1983b) is preferable to the 30 m SE-30 capillary column used by Harless et al. (1980). The HRMS technique used by Harless et al. (1980) is expected to provide a better resolution of components than the LRMS. The method of Harless et al. (1980) was suitable for the determination of ppt levels of TCDD in soils.

3.3.2.4. BIOLOGICAL MEDIA -- In this section, the sampling and analysis of PCDDs in a number of media, namely, blood, urine, fish, egg, gelatin, liver, milk, cream, lean and adipose tissue, grain, grass, leaves, vegetables and sawdust, will be discussed in general.

3.3.2.4.1. Sampling Methods -- Only a limited systematic study has been performed on the methods of sample collection for the different biological media. A review of available literature reveals certain facts that should be considered during sample collection. The concentration of PCDDs in blood is ~2-3 orders of magnitude lower than their concentrations in adipose tissue (Firestone et al., 1979). There is also evidence in several species that the accumulation of TCDD in liver tissue is higher than in adipose tissue (Section 7.2.). Liver is also preferable because its lipid content is lower than adipose tissue (samples with high lipid content are more difficult to extract and clean up). One of the most convenient sampling media that does not require sacrificing or surgically removing the tissue is milk. Because of the high lipid content of milk, PCDDs are expected to be accumulated in this medium (Langhorst and Shadoff, 1980).

The dry solid samples, such as rice grain, grass, vegetables and sawdust can be collected in polyethylene bags. Samples should be frozen in dry ice during transportation and should be stored in a freezer (-18°C) until analyzed (Jensen et al., 1983). However, it has been reported that tissue samples stored in linear polyethylene bottles sorbed ~2% of added $^{14}C$-DDT overnight and the sorbed DDT could not be washed out from the bottle (Albro, 1979). Similar absorption of 2,3,7,8-TCDD on polyethylene bags or bottles may take place. The collection of samples in clean glass jars sealed with screw caps lined with Teflon or acetone-washed aluminum foil (dull side down) is preferable (Brumley et al., 1981). The sample should be transported at 4°C and frozen until analysis.

3.3.2.4.2. Analysis -- Numerous analytical methods are available for the analysis of samples in this category (NRCC, 1981a; Crummett, 1983; Rappe et al., 1984; Smith et al., 1984). The acid/base and neutral extractions procedures are available. Neutral extraction procedures are preferred over acid/base procedures since the latter may decompose the higher PCDDs. The analytical methods for the determination of PCDDs in three typical media, namely, fish and lean tissue, adipose tissue, and milk, will be discussed here. In choosing the analytical methods, the results of the study of Brumley et al. (1981) have been given due considera- tion.

Fish and other lean tissue samples should be ground to obtain a homogeneous sample. The homogenized sample should be blended with anhydrous sodium sulfate until a free-flowing powder is obtained. The mixture should be packed into a glass column and extracted with methylene chloride. The extract should be first cleaned through a dual-column system of silica, concentrated sulfuric acid in silica, and sodium hydroxide in silica, followed by a second dual-column system of silver nitrate on silica and basic alumina. The PCDD fractions should then be cleaned up by normal phase silica HPLC, followed by reverse-phase (Zorbax-ODS) HPLC. This extraction and clean-up method is a combination of procedures employed by Huckins et al. (1978) and Lamparski et al. (1979), and is expected to provide a better method for the analysis of PCDDs in lean tissue samples.

Recently, an interlaboratory round robin study to estimate the reliability of data on the determination of 2,3,7,8-TCDD levels in fish and other aquatic species was conducted (Ryan et al., 1983). No significant differences in the determined concentration of 2,3,7,8-TCDD in these species occurred from methods differing in the use of digestion or extraction technique, HRMS or LRMS, and isomer specific or nonspecific separation. The relative standard deviations in three fish samples analyzed by seven laboratories varied between 14 and 25%. This study indicated the necessity for the use of an internal standard to obtain precise results.

Thawed adipose tissue samples should be ground with anhydrous sodium sulfate (8 g $Na_2SO_4$/g fat) in a mortar and pestle to remove excess moisture. The homogenized sample should be extracted with chloroform-methanol (2:1) in a blender. The methanol should be removed from the extract by adding aqueous KCl. The chloroform layer should then be subjected to the clean-up procedures. For the cleanup of the chloroform extract, the method described for lean tissue should be followed. The extraction and clean-up method described is a combination of procedures employed by Hass et al. (1978) and Lamparski et al. (1979). However, hexane-acetone (1:2) was used by Ryan and Williams (1983) in extracting 2,3,7,8-TCDD from human adipose tissue.

The milk samples should be mixed with sodium oxalate and ethanol and the solution extracted with ethyl ether-hexane (1:1.4). The ether-hexane extract should be dissolved in hexane and the clean-up procedure described for lean tissue should be followed. For the extraction and clean-up method, a combination of procedures employed by O'Keefe et al. (1978) and Lamparski et al. (1979) may be employed.

3.3.3. **Bioanalysis of PCDDs.** There are currently three methods for the bioanalysis of PCDDs, namely, radioimmunoassay (Albro et al., 1979; McKinney et al., 1981), AHH induction assay (Bradlaw and Casterline, 1979) and a cytosol receptor assay (Hutzinger et al., 1981; Sawyer et al., 1983). All of these methods are in the developmental stage and are neither specific for PCDDs nor are sensitive enough at low levels. The advantages of these methods are that they are inexpensive and quick compared with chemical analytical methods. Therefore, these methods have some potential for high volume screening of samples for the presence of PCDDs, but should not be used as substitutes for chemical analysis.

3.3.4. **Critique of Sampling and Chemical Analysis.** The greatest weaknesses that persist in the determination of PCDD levels in environmental samples are the lack of data for validating the accuracy of sample collection, transportation and storage procedures. The lack of representativeness of samples during collection, loss of sample by sorption on container walls or photodecomposition during transportation and storage, and contami- nation of the sample by collection equipment or sample containers can all cause errors, particularly in samples with very low residue levels. However, no comprehensive study has been done to provide enough guidance in the sampling procedures.

There are several possible points of weakness in the analytical methods as well. Although some validation data are available for the overall recovery of 2,3,7,8-TCDD in fortified matrices, these data, as shown in Table 3-6, may not represent the true recoveries, since it is difficult if

TABLE 3-6

Some Published Method Validation Data for 2,3,7,8-TCDD Recovered from Fortified Matrices and Determined by GC/MS

| m/e Values | Matrix | TCDD Level of Fortification, ng/kg$^{-1}$ |  | Number of Replicates | Mean % Recovery with S.D. |  | Reference |
|---|---|---|---|---|---|---|---|
|  |  | Native | Isotope $^{13}C$, ($^{37}Cl$) |  | Native | Isotopes |  |
| 320, 322, 335 | human milk | 2.6 | 166 | 8 | 25 ± 7 | 37 ± 19 | Langhorst and Shadoff, 1980 |
| 320, 322, 324 | soil | NA | 100[a] | 6 | NA | 87 ± 15 | Hummel, 1977 |
| 320, 322, 324 | soil | 10 | NA | 28 | 87 ± 17 | NA | DiDomenico et al., 1980a |
| 320, 322, 324, 335 | soil | 50 | ,[b] | 8 | 99.2 ± 5 | 59.8 | Lamparski and Nestrick, 1980 |
| 320, 322, 328 | fish, liver | 0-125 | 1000[a] | 17 | ±15[c] | 86 ± 15 | Harless et al., 1980 |
| 320, 322, 328 | human milk | 0-5 | 250[a] | 13 | ±38[c] | 68 | Harless et al., 1980 |
| 320, 322, 328 | water, sediment | 0.01-1000 | 250[a] | 14 | ±16[c] | 87 | Harless et al., 1980 |
| 320, 322, 328, 329 | water, sediment | 0.7-65 | 66 | 12 | 85-100 (±8-±17) | 71-87 (±12-±21) | O'Keefe et al., 1978 |
| 320, 322, 328 | water, sediment | 2 | NA | 3 | 83.3 | NA | Mahle et al., 1977 |
| 320, 322, 328 | water, sediment | NA | 625[a] | 4 | NA | 64 | Mahle et al., 1977 |
| 320, 322, 328, 329 | bovine feed | 13-200 | 390-1000 | 16 | 80-100 (±5-±18) | 77-105 (±9-±18) | O'Keefe et al., 1978 |
| 320, 322, 328 | liver | 20 | 1000 | 9 | 34 ± 7 | 27 ± 5 | Baughman and Meselson, 1973 |

TABLE 3-6 (cont.)

| m/e Values | Matrix | TCDD Level of Fortification, ng/kg$^{-1}$ |  | Number of Replicates | Mean % Recovery with S.D. |  | Reference |
|---|---|---|---|---|---|---|---|
|  |  | Native | Isotope $^{13}$C, ($^{37}$Cl) |  | Native | Isotopes |  |
| 320, 322, 324 | carrots | 0.5-1.0 | NA | 20 | 64.5-66.6 (±18.9-±25.5)$^{d}$ | NA | Cavallaro et al., 1980b |
| 320, 322, 324 | beets | 0.5-1.0 | NA | 20 | 60.8-79.8 (±17-±17.7)$^{d}$ | NA | Cavallaro et al., 1980b |
| 320, 322, 324 | spinach | 0.5-1.0 | NA | 20 | 46.6-67.7 (±14.2-±24.7)$^{d}$ | NA | Cavallaro et al., 1980b |

[a] Indicates publishing author's recovery data was converted from ng to ppt or from ppt to %.

[b] Plus indicates fortified with isotope but amount not specified clearly.

[c] These data indicate the mean % accuracy for TCDD obtained with quality assurance samples.

[d] Number in the bracket represents the % variation experienced; unclear as to how calculations were made.

NA = Not added; SD = Standard deviation

not impossible to incorporate the internal standard in the same physical/chemical form in the sample matrix as the PCDDs. This situation weakens the reliability of much of the analytical data on PCDD levels in various matrices.

The recovery of the overall analytical procedures is normally done by measuring the recovery of internal standards such as $^{37}Cl_4$-TCDD and $^{13}C$-TCDD. Methods that used internal standards that exceeded the native TCDD by 50-2500 times are at best questionable. Also, the recovery data based on one internal standard to correct for another congener or another isomer, such as 1,2,3,4-TCDD for OCDD or 2,3,7,8-TCDD, may be questionable in view of the fact that recovery and response factors may vary between congeners and isomers. This could cause serious problems with the determined detection limits.

Despite some rigorous criteria (Harless et al., 1980) that may be used for positive identification of 2,3,7,8-TCDD (assuming that the GC column resolves 2,3,7,8-TCDD from other TCDDs), false positive results have been obtained under certain conditions. A collaborative study conducted by the U.S. EPA exemplifies this point. Of the total of 20 unspiked samples in this study, 10 gave false positive results (Crummett, 1980). In a recent method validation study by the U.S. EPA (Gross et al., 1981), 2,3,7,8-TCDD levels <9 ppt could not be detected with accuracy. Clearly, there is a need for more exhaustive examination for potential interferences that may cause false positive results.

Another major factor limiting the research in the field is the shortage or lack of availability of individual isomers. Unless the authentic compounds are available, analytical data developed for one isomer on the basis of the response factor of another isomer will remain largely questionable.

## 3.4. SUMMARY

The solubility of 2,3,7,8-TCDD in water is 0.2 µg/ℓ. This congener and the other three PCDDs are more soluble in aromatic solvents than aliphatic solvents. The PCDDs are relatively stable in the environment and they start to decompose at temperatures >500°C.

The general method for the determination of these compounds in different sample matrices consists of a solvent extraction procedure to transfer the PCDD residue into the solvent(s), followed by $H_2SO_4$ and base washes to remove the excess lipid and other impurities from the solvent extract. The extract is then subjected to two liquid chromatographic clean-up procedures. The cleaned up extract is finally analyzed for the PCDDs by a GC/MS method. All the possible GC/MS combinations, namely, HRGC-LRMS, LRGC-LRMS, LRGC-HRMS and HRGC-HRMG, have been used. However, if an unequivocal identification and quantification of several specific isomers is required, two methods are suitable. One involves a 55 m Silar 10C glass capillary or a 60 m SP-2330 fused silica column in combination with LRMS. Another method using RP-HPLC and normal phase HPLC separation in combination with LRMS has been found to be satisfactory.

# 4. PRODUCTION, USE, SYNTHESIS, ENVIRONMENTAL SOURCES AND ENVIRONMENTAL LEVELS

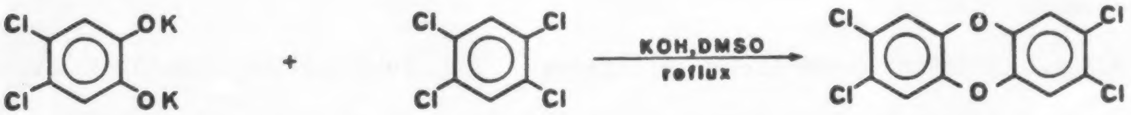
## 4.1. PRODUCTION AND USE

PCDDs including the four compounds discussed in this document are not commercially produced. Rather, these compounds are formed as trace amounts of unwanted impurities in the manufacture of other chemicals, primarily chlorophenols and their derivatives. There is no known technical use for the PCDDs (Rappe et al., 1979). The amount of total PCDDs entering the Canadian environment/year has been speculated to be ~3300 pounds and 75% of this amount has been estimated to be due to OCDD alone (NRCC, 1981a).

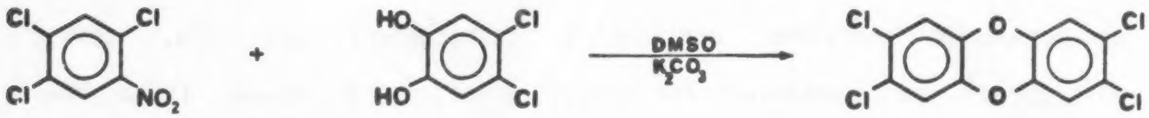
## 4.2. SYNTHESIS

Although the PCDDs are not commercially produced, some of these compounds have been synthesized according to reactions discussed below (U.S. EPA, 1980a).

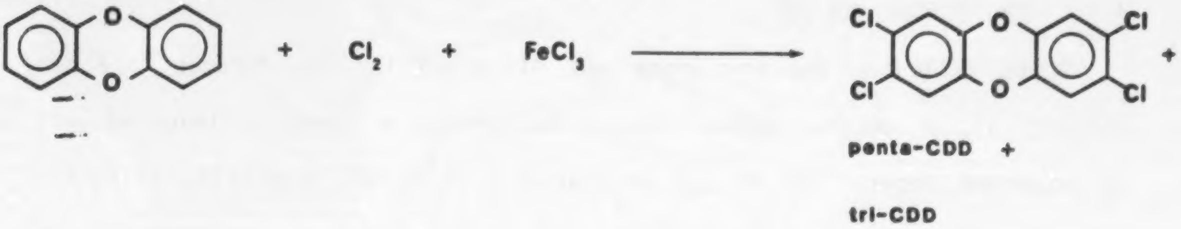
4.2.1. Reaction of Dichlorocatechol Salts with 1,2,4,5-Tetrachlorobenzenes in DMSO. This general reaction has been used to synthesize 2,3,7,8-TCDD according to the reaction scheme shown below:



The yield of 2,3,7,8-TCDD by this reaction is low (Kende et al., 1974). A better method is the reaction of o-dichlorocatechol with 3-nitro-2,5,6-trichlorobenzene as shown below (Gray et al., 1976):



4.2.2. **Substitution Reaction.** The following substitution reactions have been used for the synthesis of 2,3,7,8-TCDD:



The yield of 2,3,7,8-TCDD by this reaction has been reported to be low (U.S. EPA, 1980a). However, when the chlorination of the unsubstituted dibenzo-p-dioxin was conducted without the $FeCl_3$, the yield of 2,3,7,8-TCDD was reported to be 40-50% (U.S. EPA, 1980a). The substitution of dibenzo-p-dioxin with 2,3-dichlorodibenzo-p-dioxin in the presence of $FeCl_3$ and iodine, on the other hand, reportedly also produced a high yield (41%) of 2,3,7,8-TCDD (Kende et al., 1974).

4.2.3. **Photoproduction.** Small amounts of mixtures of lower PCDDs have been produced by the UV irradiation of OCDD (Buser, 1979). For example, a mixture of tri-, penta-, hexa- and hepta-CDD has been produced by this method.

4.2.4. **Ullmann Condensation Reactions.** The condensation reactions as shown in Figure 4-1 have been used for the synthesis of tetra- and hexa-CDD.

The yield of the desired products by the condensation reactions are not always satisfactory because of other competing reactions. Examples of some of these competing reactions are condensation with Cl atoms meta to a hydroxyl group, condensation of Cl atoms para to the hydroxyl group, dechlorination reactions, and Smiles rearrangement (U.S. EPA, 1980a). Although the best conditions for dioxin formation are unknown, it has been speculated that a temperature of 180-400°C, a pressure of >1 atmosphere

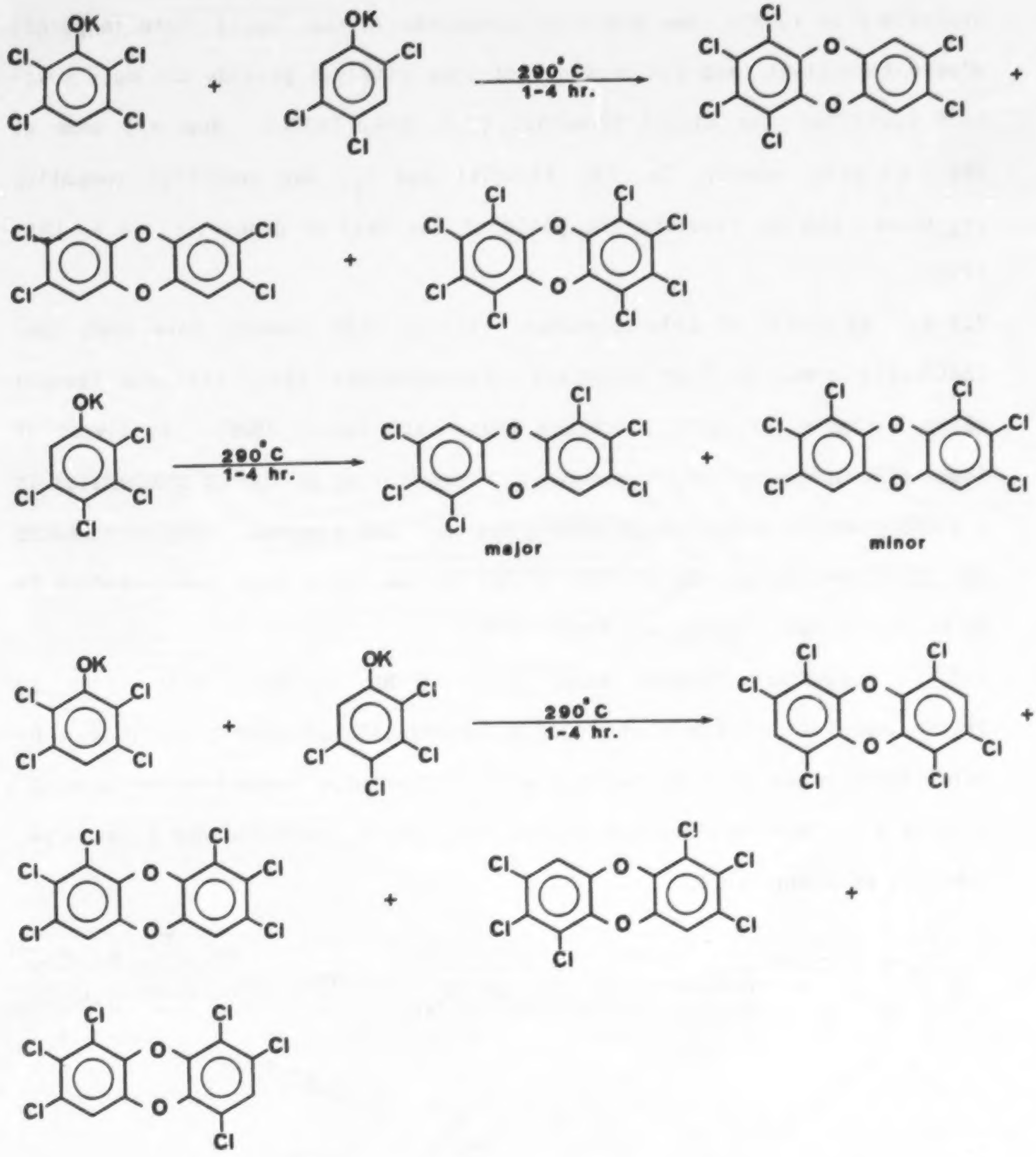


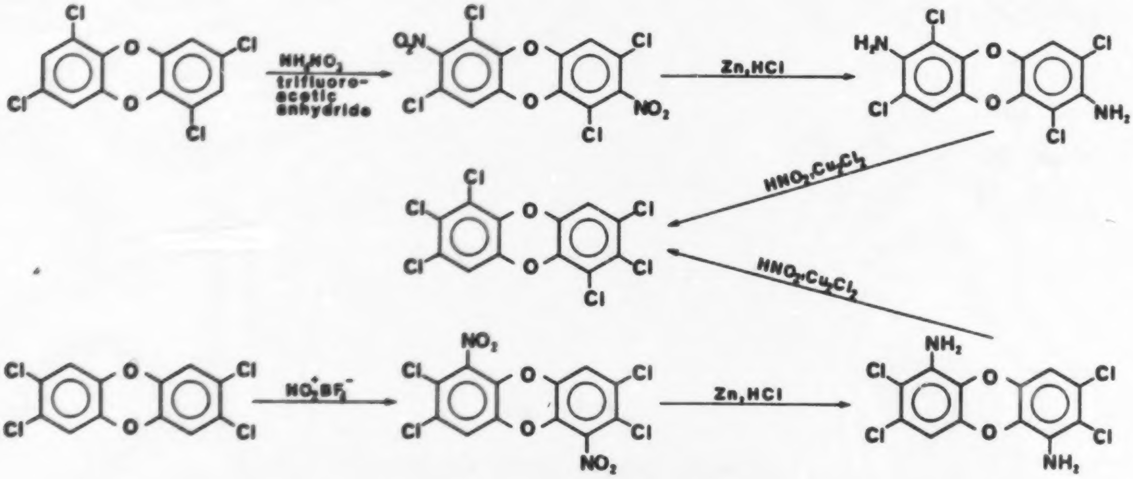
FIGURE 4-1

Ullmann Condensation Reactions

(necessary to retain some precursor compounds in the liquid state to permit dioxin formation), and the presence of some catalyst provide the most suitable conditions for dioxin formation (U.S. EPA, 1980a). However, some of the catalysts, namely, Cu, Fe, Al-salts and $I_2$, may encourage competing reactions, thereby reducing the yield of the desired product(s) (U.S. EPA, 1980a).

**4.2.5. Pyrolysis of Chlorophenates.** All 22 TCDD isomers have been synthetically prepared from different chlorophenates (di-, tri- and tetra-) using a simple pyrolysis procedure (Buser and Rappe, 1980). Pyrolyses of these chlorophenates were conducted by placing 1 mg of the chlorophenates in a glass reaction tube plugged with glass wool and alumina. They were heated for 30-60 minutes at 300°C. The yields of the TCDDs have been reported to be in the µg range (Buser and Rappe, 1980).

**4.2.6. Conversion Through Nitration.** It has recently been shown by Oliver and Ruth (1983) that 1,2,3,6,7,8-hexachlorodibenzo-p-dioxin can be selectively prepared from two synthetic routes each consisting of dinitration of a tetrachlorodibenzo-p-dioxin, followed by reduction and a Sandmeyer reaction as shown below:



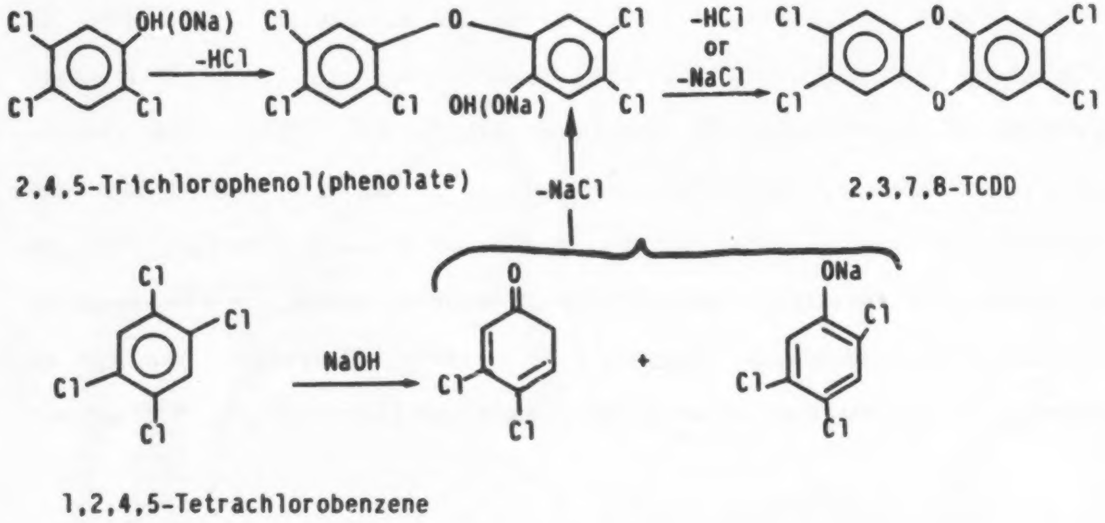
The recovery of 1,2,3,6,7,8-HxCDD was excellent by this method.

## 4.3. ENVIRONMENTAL SOURCES

The sources of PCDDs and particularly 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD in the environment can be broadly divided into five categories, namely, manufacturing processes, municipal incinerations, other combustion processes, chemical disposal sites and photochemical processes. The last source may not significantly contribute to PCDD contamination in the environment. Each of these categories is discussed individually in the following subsections.

**4.3.1. Manufacturing Processes.** PCDDs are generally produced during the production of chlorinated phenols, during the production of chemicals utilizing the chlorophenols (i.e., 2,4,5-T and 2,4-D) and in various industrial incinerators where materials containing chlorinated phenol and polychlorinated diphenyl ethers are incinerated.

4.3.1.1. PRODUCTION OF CHLOROPHENOLS -- PCDDs are formed as by-products during the manufacture of chlorophenols. Chlorophenols are produced by two processes, the chlorination of phenols and the alkaline hydrolysis of the appropriate chlorobenzenes. Hypothetically, both processes can lead to the formation of PCDDs according to the mechanism depicted below (U.S. EPA, 1980a):



Similarly, HxCDDs are formed during the manufacture of tetrachlorophenols by the above reaction process. PCDDs are also expected to be formed during the hydrolytic production of polychlorinated benzenes. The amounts of PCDDs in commercial chlorophenols vary according to manufacturing process and conditions. The levels of TCDDs, PeCDDs and HxCDDs found in different chlorophenols have been shown in Table 4-1. It can be seen from Table 4-1 that the specific isomers of the TCDDs, PeCDDs and HxCDDs have not always been identified in the products. However, 2,3,7,8-TCDD has been identified in commercial trichlorophenols (Table 4-1). On the other hand, 2,3,7,8-TCDD is not produced in the manufacture of PCP (Buser and Rappe, 1978). The main HxCDD isomers produced during the manufacture of PCP are 1,2,4,6,7,9-, 1,2,3,6,8,9- and 1,2,3,6,7,8-HxCDD present in a ratio of 1:4:5 (Buser, 1979). However, the composition and quantities of PCDDs in PCP may vary widely from batch to batch and manufacturer to manufacturer, depending on the manufacturing processes.

The annual world production of chlorophenols is estimated to be ~150,000 tons (Rappe et al., 1979). U.S. production figures for di- and tetrachlorophenols are not available. However, the 1977 estimated figures indicate that the annual production capacity for PCP in the United States was 53 million pounds (U.S. EPA, 1980a). Canadians manufacture ~4000 tons of chlorophenols annually with the total release inventory to the environment estimated at >1365 tons/year (Environmental Canada, 1984). The chlorophenols are used as fungicides, herbicides, slimacides, bactericides and intermediates in the production of chlorinated phenoxy acid herbicides in agriculture and forestry. The antiseptic, hexachlorophene, is also prepared from 2,4,5-trichlorophenol (Rappe et al., 1979). Therefore, the use or presence of contaminated chlorophenols in facilities such as chlorophenol

TABLE 4-1

Levels of Tetra-, Penta- and Hexa-chlorodibenzo-p-dioxins Reported in Chlorophenols and a Few Pesticides Originating from Chlorophenols

| Compound | Chlorodibenzo-p-dioxin (-CDD) level, ppm | | | No. Contam.[a] / No. Tested | Reference |
|---|---|---|---|---|---|
| | Tetra- | Penta- | Hexa- | | |
| o-Chlorophenol | ND | ND | ND | 0/1 | Firestone et al., 1972 |
| | 0.037[b] | NR | NR | several samples | Anonymous, 1979 |
| 2,4-Dichlorophenol | ND | ND | ND | 0/1 | Firestone et al., 1972 |
| 2,6-Dichlorophenol | ND | ND | ND | 0/1 | Firestone et al., 1972 |
| 2,4,5-TCP[c] | ND-6.2 (2,3,7,8-)[d]<br>ND-0.3 (1,3,6,8-) | ND-1.5 | ND | 3/4 | Firestone et al., 1972 |
| 2,4,6-TCP | 49 (1,3,6,8-) | ND | ND | 1/1 | Firestone et al., 1972 |
| 2,4,5-TCP (Na salt) | 1.40 (2,3,7,8-) | ND | ND | 1/2 | Firestone et al., 1972 |
| TCP (unspecified) | ND | NR | ND-<10 | 4/6 | Woolson et al., 1972 |
| 2,3,4,6-Tetrachlorophenol | ND | ND | ND-29 | 2/3 | Firestone et al., 1972 |
| | 0.7 | 5.2 | 9.6 | NA | Rappe et al., 1978 |
| | NR | NR | 6 | 1/1 | Buser, 1975 |
| Tetrachlorophenol (unspecified) | ND | NR | ND-<100 | 3/3 | Woolson et al., 1972 |
| PCP[e] | ND | ND | 0.17-39 | 6/6 | Firestone et al., 1972 |
| | ND | NR | ND-<100 | 10/11 | Woolson et al., 1972 |
| | NR | NR | 9 | 1/1 | Buser, 1975 |
| | ND | NR | 9-27 | several samples | AWPI, 1977 |
| | ND | NR | 0.02-42 | 2/2 | Villaneuva et al., 1973 |
| | ND | ND | 0.03-10 | 12/13 | Buser and Bosshardt, 1976 |

TABLE 4-1 (cont.)

| Compound | Chlorodibenzo-p-dioxin (-CDD) level, ppm | | | No. Contam.[a] / No. Tested | Reference |
|---|---|---|---|---|---|
| | Tetra- | Penta- | Hexa- | | |
| PCP (cont.) | NR | NR | ND-2 | several samples | Dow, 1978 |
| PCP (Na salt) | ND | ND | 14-20 | 2/2 | Firestone et al., 1972 |
| | 0.06-0.4 | ND-0.08 | ND-6.8 | 6/6 | Buser and Bosshardt, 1976 |
| 2,4-D (-DB, -DP)[f] | ND | ND | ND-<10 | 1/28 | Woolson et al., 1972 |
| 2,4-D and 2,4,5-T mixtures (formulated products) | ND | ND | ND | 0/10 | Norstrom et al., 1979 |
| 2,4-D (acid, esters, and amines) | ND-8.73[g] (1,3,6,8-/ 1,3,7,9-) | NR | NR | 28/58 | Cochrane et al., 1981 |
| 2,4-D (acid, esters, and amines) | D (1,3,6,8-) | NR | NR | 2/30 | Thomas, 1980a; Harless, 1981 |
| 2,4,5-T[h] | ND-<100 | NR | ND-<100 | 23/42 | Woolson et al., 1972 |
| 2,4,5-T (acid, esters, and formulated products) | 0.010-0.080 (2,3,7,8-) | NR | NR | 12/30 | ACP, 1980 |
| Silvex® | ND-<10 | NR | ND | 1/7 | Woolson et al., 1972 |
| Agent Orange (1:1 mixture of butyl esters of 2,4-D and 2,4,5-T) | 1.98[i] (2,3,7,8-) | NR | NR | 490/490 | Young, 1983 |
| Agent Purple (5:3:2 mixture of n-butyl 2,4-D, n-butyl 2,4,5-T and iso-butyl 2,4,5-T) | 32.8[i] (2,3,7,8-) | NR | NR | NR | Young, 1983 |

[a] These are the ratios of the number of samples contaminated with any chlorodioxins to the number of samples tested.
[b] 2,3,7,8-isomer detected but not quantified
[c] TCP: trichlorophenol
[d] These indicate specific dioxin concentrations.
[e] PCP: pentachlorophenol
[f] These are dichlorophenoxy-acetic, -butyric acid and -propionic acid.
[g] The isomers could not be separated.
[h] This is 2,4,5-trichlorophenoxy acetic acid.
[i] This is an average value.
ND = Not detected; NR = Not reported; D = detected; NA = Not available

and pesticide/herbicide plants, cooling towers, pulp and paper industry, incinerators and disposal sites are potential exposure areas for PCDDs (Josephson, 1983).

The locations of current and former producers and formulators of chlorophenols are presented in Table 4-2. The inclusion of the locations of the former producers has been judged necessary for the identification of past sources of contamination that may present an environmental hazard in the future (i.e., airborne contaminated dust particles) because of the environmental persistence of 2,3,7,8-TCDD (Chapter 5).

4.3.1.2. PRODUCTION OF CHLOROPHENOL DERIVATIVES -- PCDDs have been detected also as contaminants produced during the manufacture of commonly used chlorophenol derivatives, such as 2,4-D, 2,4,5-T and hexachlorophene by mechanisms hypothesized to be similar to those discussed in the case of chlorophenols. The amounts of 1,3,6,8- and 1,3,7,9-TCDD in commercial iso-octyl-, mixed butyl- and propylene glycol butyl ether ester of 2,4,-D varied from nondetectable to 8.7 mg/kg (Cochrane et al., 1981). Agent Orange, which is a 1:1 mixture of the butyl esters of 2,4-D and 2,4,5-T, has been shown to contain 2,3,7,8-TCDD in quantities in the range of 0.1-47 μg/g (Rappe et al., 1979). The 2,3,7,8-TCDD impurity in Agent Orange has been shown to originate from 2,4,5-T. The mean levels of 2,3,7,8-TCDD in Agent Orange and Agent Purple (50% n-butyl 2,4-D, 30% n-butyl 2,4,5-T and 20% isobutyl 2,4,5-T) preparations used in the 1960s were shown to be 1.98 and 32.8 ppm, respectively (Young, 1983). Efforts were made during the 1970s to control and minimize the formation of 2,3,7,8-TCDD and, at the present time, all the producers claim that their products contain <0.1 μg/g of 2,3,7,8-TCDD (Rappe et al., 1979).

## TABLE 4-2

## Locations of Companies that have been Major Producers and Formulators of Chlorophenols and Their Derivatives[a]

| Chemical | Producer |
|---|---|
| 2,4-D Acid and Esters | Alco Chemical Corp., Philadelphia, PA |
| | *Amvac-Chemical Corp., Los Angeles, CA[b] |
| | Chempar, Portland, OR |
| | *Diamond Shamrock Corp., Tuscaloosa, AL<br>Cleveland, OH |
| | Diamond Alkali, Newark, NJ |
| | *Dow Chemical, U.S.A., Midland, MI |
| | Fallek-Lankro Corp., Tuscaloosa, AL |
| | GAF, Linden, NJ |
| | *Guth Corp., Hillside, IL |
| | Hercules, Inc., Jacksonville, AR |
| | Imperial, Inc., Shenandoah, IA |
| | Miller Chemical, Whiteford, MD |
| | Monsanto, Co., Sauget, IL |
| | North American Phillips Corp., Kansas City, KS |
| | *PBI-Gordon Corp., Kansas City, KS |
| | Rhodia, Inc., Portland, OR<br>St. Paul, MN<br>St. Joseph, MO |
| | *Rhone-Poulenc, Inc., Portland, OR |
| | *Riverdale Chemical Co., Chicago Heights, IL |
| | Rorer-Amchem, Fremont, CA<br>St. Joseph, MO |
| | Thompson Chemical, St. Louis, MO |
| | Union Carbide Corp., Ambler, PA |
| | *Velsicol Chemical Corp., Beaumont, TX<br>Bayport, TX |
| | Vertac, Inc., Jacksonville, AR |
| | Woodbury, Orlando, FL |
| 2,4,5-T | Chempar, Portland, OR |
| | Diamond Shamrock, Cleveland, OH |
| | Dow Chemical, U.S.A., Midland, MI |
| | Hoffman-Taft, Inc., Springfield, MO |
| | Monsanto Co., Sauget, IL |
| | North American Phillips Corp., Kansas City, KS |
| | PBI-Gordon Corp., Kansas City, KS |
| | Rhodia Inc., Portland, OR<br>St. Joseph, MO |
| | *Riverdale Chemical Co., Chicago Heights, IL |
| | Rorer-Amchem, Ambler, PA<br>Fremont, CA |

TABLE 4-2 (cont.)

| Chemical | Producer |
|---|---|
| 2,4,5-T (cont.) | Rorer-Amchem, St. Joseph, MO<br>Jacksonville, AR<br>Thompson Chemical, St. Louis, MO<br>Union Carbide Corp., Fremont, CA<br>St. Joseph, MO<br>Ambler, PA<br>Vertac, Inc., Jacksonville, AR |
| 2,4,5-T derivatives<br>Silvex esters and salts | Dow Chemical U.S.A., Midland, MI<br>Hercules, Inc., Jacksonville, AR<br>North American Phillips Corp., Kansas City, KS<br>*Riverdale Chemical Co., Chicago Hts., IL<br>Vertac, Inc., Jacksonville, AR |
| Ronnel | *Dow Chemical U.S.A., Midland, MI |
| Erbon | *Dow Chemical U.S.A., Midland, MI |
| Hexachlorophene | Givaudan Corp., Clifton, NJ |
| 2,4,5-TCP and salts | Diamond Shamrock Corp., Cleveland, OH<br>Dow Chemical, U.S.A., Midland, MI<br>GAF Corp., Linden, NJ<br>Hercules, Inc., Jacksonville, AR<br>Hooker Chemical, Niagara Falls, NY<br>Merck and Co., Inc., Rahway, NJ<br>Nalco Chemical Co., Chicago, IL<br>North Eastern Pharmaceuticals, Verona, MO<br>Roberts Chemical, Inc., Nitro, WV<br>Rhodia, Inc., Monmouth Junction, NJ<br>Vertac, Inc., Jacksonville, AR |
| 2,3,4,6-Tetrachlorophenol | Dow Chemical U.S.A., Midland, MI<br>Sanford Chemical, Port Neches, TX |
| PCP and salts | J.H. Baxter and Co., San Mateo, CA<br>Dow Chemical U.S.A., Midland, MI<br>ICC Industries, Inc., Dover, OH<br>Monsanto Co., Sauget, IL<br>Nalco Chemical Co., Chicago, IL<br>*Reichhold Chemical, Inc., Tacoma, WA<br>Sanford Chemical, Port Neches, TX<br>*Vulcan Materials Co., Wichita, KS |

[a] Sources: U.S. EPA, 1980a; SRI, 1982; USITC, 1982

[b] Company names indicated with an asterisk are the major producers of chlorophenols and their derivatives at the present time.

As can be seen from Table 4-1, 2,4-D, 2,4,5-T and their formulated products may contain other PCDDs in addition to TCDDs. It has also been reported that Agent Orange and 2,4,5-T samples used during the Vietnam conflict contain other PCDDs at levels similar to that of 2,3,7,8-TCDD. Agent Orange and European 2,4,5-T formulations from the 1960s, on the other hand, may contain primarily 2,3,7,8-TCDD and only minor amounts of other PCDDs (Rappe et al., 1979). The average 2,3,7,8-TCDD contents in Agent Orange and Agent Purple given in Table 4-1 refer to these materials manufactured in the 1960s.

Hexachlorophene is prepared from the same starting material as 2,4,5-T, namely, 1,2,4,5-tetrachlorobenzene. Because of additional purification, however, the level of 2,3,7,8-TCDD in this product has been reported to be $\leq$0.03 μg/g (Rappe et al., 1979).

The locations of current and former producers of chlorophenol derivatives have been shown in Table 4-2.

4.3.1.3. CONTAMINATED MANUFACTURING EQUIPMENT -- Production trains are often used for the production of chemicals whose manufacture necessitates the use of similar process equipment. In the manufacture of chemicals on a production train previously contaminated with PCDDs, both the products and waste generated can be contaminated with PCDDs. Thus, the manufacture of 2,4-D, which otherwise was not expected to be contaminated with 2,3,7,8-TCDD, did indeed contain 2,3,7,8-TCDD because the equipment used had been employed previously to produce 2,4,5-T, and the equipment remained contaminated with 2,3,7,8-TCDD (Federal Register, 1980a).

4.3.1.4. DIPHENYL ETHER HERBICIDES -- The presence of TCDDs, PeCDDs and HxCDDs as contaminants in diphenyl ether herbicides was reported by Yamagishi et al. (1981). The source of PCDDs in these herbicides was speculated to be the trichlorophenol used in their production. The concentrations of the two major impurities, TCDDs and PeCDDs, in commercial formulations were ~150 and 30 ppm, respectively. The isomeric distribution of TCDDs showed that the major components were 1,3,6,8- and 1,3,7,9-isomers. The isomer 2,3,7,8-TCDD was not detected in the commercial products.

4.3.1.5. INCINERATION OF SELECTED INDUSTRIAL WASTES -- The combustion of a variety of chlorinated hydrocarbons has been shown to produce PCDDs (Tiernan et al., 1982a). The formation of PCDDs would likely occur in incinerators operating at 750-900°C; chlorophenols are probably the precursors of PCDD formation. At temperatures >1200-1400°C and residence time of <1 second, PCDDs are likely to decompose and these compounds are not expected to form (Junk and Richard, 1981). From kinetic and thermodynamical considerations, Shaub and Tsang (1983) estimated that 99.99% gas phase dissociation of tetrachlorodibenzo-p-dioxins at 727°C may require ~15 minutes, while the same decomposition at 977°C may require <1 second.

In an industrial boiler in the United States where PCP was known to have been burned, Rappe et al. (1983b) reported ~5 ppm PCDDs in the bottom and baghouse ash. More than 90% of the PCDDs were lower chlorinated congeners than OCDD and only a small amount of 2,3,7,8-TCDD was detected. Soot analysis of a recent transformer fire in Binghamton, NY, in February, 1981, revealed that 2,3,7,8-TCDD (0.6 ppm) and 1,2,3,7,8-PeCDD (2.5 ppm) were the dominating isomers of the PCDDs formed (Buser and Rappe, 1983; Rappe et al., 1983b). The origin of the PCDDs was probably the chlorobenzenes in the transformer oil (Buser, 1979). The analysis of wipe tests from a garage adjacent to this site did reveal the presence of PCDDs before cleaning the garage. Following the cleanup, no contamination was found (Tiernan et al., 1982b; Tiernan, 1983). Therefore, it is important to recognize the possibility of production of PCDDs and PCDFs in fires involving PCB and chlorobenzene transformers.

4.3.2. **Municipal Incinerators.** PCDDs have been detected both in the fly ash and air particulate matter from municipal incinerators by several investigators in Canada, Europe and the United States. The particulate matter forming the emissions (air particulates) has a 10-fold greater concentration of PCDDs than the precipitated material (fly ash) (Lustenhouwer et al., 1980). The concentration of total TCDDs, PeCDDs and HxCDDs in the fly ash from a variety of municipal incinerators in Canada, Europe and the United States have been studied by several authors (Eiceman et al., 1979, 1980; Nestrick et al., 1982; Karasek et al., 1982; Bumb et al., 1980; Buser and Bosshardt, 1978; Tiernan et al., 1982a; Taylor et al., 1983). The TCDD isomer known to be the most toxic (i.e., 2,3,7,8-TCDD) was either not detected or detected at a low level. The quantities emitted in incinerators vary, probably because of differing efficiencies, and since few municipal incinerators have been reliably characterized for PCDD/PCDF emissions over extended time intervals, the data base is still inadequate. Whereas Bumb et al. (1980) and Buser and Rappe (1980) detected 0.4 ng/g of 2,3,7,8-TCDD in the fly ash from a United States municipal incinerator, the U.S. EPA concluded that emissions from five municipal waste combustors did not present a public health hazard for residents living in the immediate vicinity (CEQ, 1981). Evaluation of stack emissions of PCDDs have to be based on the amount of dioxins in both the flue gas condensate followed by an effective absorption or adsorption step (Ballschmiter et al., 1984). PCDDs have been detected in the emissions of some municipal waste incinerators in Europe (Gizzi et al., 1982; Benfenati et al., 1983; Taylor et al., 1983; Olie et al., 1982, 1983; Lustenhouwer et al., 1980; Barnes, 1983). Observations on PCDD emissions from an industrial boiler have been discussed in Section 4.3.1.5. (Rappe et al., 1983b).

In a study of municipal fly ash conducted between a single incinerator in the United States and one in Europe, Lamparski and Nestrick (1980) detected at least 14 of the 22 possible TCDD isomers. Although the ratio of isomers to the total present were similar in both fly ashes, their absolute amounts varied by a factor $\geq 10$. It has been demonstrated by Rappe et al. (1979) that minor amounts of the highly toxic PCDD congeners, 1,2,3,7,8-PeCDD, 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD, are also formed in municipal incinerators.

4.3.3. Other Combustion Processes. Scientists from Dow Chemical Co. (Dow, 1978) reported the detection of PCDDs in particulate matter from most combustion sources. These findings led to a hypothesis which suggested that PCDDs may be formed in trace amounts from chemical reactions during the combustion of many chlorinated hydrocarbons (Bumb et al., 1980; Crummett et al., 1981). These investigators detected PCDDs including TCDDs and HxCDDs in particulate matter from municipal and industrial incinerators, in mufflers from diesel truck and passenger vehicles, from home wood-burning fireplaces and from soot and cigarette smoke. Since the trace chemistries of fire hypothesis was presented, several investigators have attempted to test it. Tiernan (1982) reported the detection of 0.65 ppb TCDD in soot from a wood-burning fireplace. Although there is general agreement regarding the production of PCDDs from the burning of wood with additional HCl and from incinerators burning chlorinated products or wastes (Tiernan et al., 1982a, Tiernan, 1983), production from the combustion of coal and hydrocarbons (such as occurs in gas burners, and auto and truck engines) has not been confirmed (NRCC, 1981a). For example, Rappe et al. (1979) concluded from their pyrolysis experiments that PCDDs are produced by the burning of very specific chemicals, such as chlorinated phenols, polychlorinated benzenes and polychlorinated diphenyl ethers. Wood pregnated with these compounds might produce PCDDs during incineration and the history of wood to be burned in fireplaces is often unknown. Junk and Richard (1981) and Kimble and Gross (1980) failed to measure TCDD above the detection limits of 1 or 1.2 ppt, respectively, from their analysis of one fly ash sample from stack emissions of a low sulfur and high-ash coal burning power plant. Recent investigations (Hailey et al., 1983; Stanley et al., 1982) also failed to detect (detection limit: flue gas, 100-700 pg/m$^3$; fly ash, 10-70 pg/g) PCDD homologues in any sample from four coal-fired power plants. Independent confirmation of "trace chemistries of fire" as proposed by Dow, U.S.A., is not yet available.

Czuczwa and Hites (1984) and Czuczwa et al. (1984, 1985) analyzed for the PCDDs and PCDFs in sediments from the Great Lakes including the sediment core from Siskiwit Lake of Isle Royale in northern Lake Superior. The sediment that came from Siskiwit Lake was used because it received only atmospheric inputs. In all cases the authors detected the flux of PCDDs and PCDFs, which began at about 1940. When this "1940 horizon" was compared with combustion trends in the last century, the authors found evidence that the combustion of synthetic chlorinated organic chemicals is the primary source of PCDDs and PCDFs. Furthermore, the authors responded that the flux of PCDDs and PCDFs to three Swiss lakes, where combustion has been extensive during the last century, increased only after the development of the chlorinated organic chemical industry. The authors also addressed the debate regarding 2,3,7,8-TCDD in coal fly ash. Reaffirming similar findings, no 2,3,7,8-TCDD was found above a detection limit of 100 ppt. These results strongly suggest that coal combustion is not a significant source of 2,3,7,8-TCDD contamination to the environment.

4.3.4. Chemical Dump Sites. At present, other potential sources of PCDDs are chemicals known to be contaminated with PCDDs but withdrawn from use and awaiting disposal, and disposal sites where chemical wastes containing PCDDs have been dumped. It has been estimated that ~11,600 metric ton/year of hazardous wastes are produced in the manufacture of chlorophenols and ~79,000 metric ton/year are produced in the manufacture of phenoxy compounds (Jett, 1982). Process wastes from the manufacture of chlorophenols and phenoxy compounds are landfilled, or injected into deep-well. Treatment wastes are frequently subjected to on-site impoundment (Jett, 1982). Recent Canadian environmental data indicate that 2,3,7,8-TCDD may be leaking into the Great Lakes from toxic dump sites (Hallett, 1984).

4.3.5. Photochemical Process. Photochemical processes can also lead to formation of PCDDs. For example, the dimerization of chlorophenols to OCDD has been studied by Crosby and Wong (1976). Lamparski et al. (1980) also reported that photolysis of PCP-treated woods may lead to the formation of PCDDs. Similarly, photochemical cyclization of predioxins (chlorinated 2-phenoxyphenols, precursors of PCDDs) can also produce PCDDs. Since predioxins are common impurities (1-5%) in commercial chlorophenols, exposure of chlorophenols containing those impurities to light may produce PCDDs (Nilsson et al., 1974).

Another photochemical process of potential environmental importance is the formation of highly toxic TCDD and PeCDD congeners from the dechlorination of higher PCDDs. However, photolysis of 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9HxCDD produced only 13% of the toxic 1,2,3,7,8-PeCDD and no 2,3,7,8-TCDD (Kim et al., 1975), while the photolysis of octa-CDD was shown to produce mainly 1,4,6,9-TCDD, 1,2,4,6,9-PeCDD and 1,2,4,6,7,8-HxCDD. Consequently, it was concluded that the most toxic isomers are not likely to be formed from the photolysis of the higher PCDDs (Buser and Rappe, 1978).

Formation of tetra- and pentachlorodibenzo-p-dioxins has been observed by the photolysis of 1,2,3,6,7,8- and 1,2,3,7,8,9-HxCDDs (Buser, 1979). There seems to be a preferential dechlorination of the HxCDDs occurring at the lateral positions flanked on both sides by adjacent chlorines (Choudhry and Hutzinger, 1984). However, formation of trace amounts of 2,3,7,8-TCDD were also observed from the photolysis of the above two isomers of HxCDDs (Buser, 1979).

## 4.4. RELATIONSHIP BETWEEN SOURCES AND CONTAMINATION IN ENVIRONMENTAL MATRICES

The potential relationship between various sources of PCDDs and the environmental matrices where these compounds have been detected (NRCC, 1981a) is depicted in Figure 4-2, which has been modified from the original reference to indicate the possible inhalation exposures from these sources.

## 4.5. ENVIRONMENTAL LEVELS

The detection of PCDD residues, particularly the residue of the four toxic PCDDs under discussion, in various environmental matrices is indicative of the potential impact that the various sources could have on the environment. However, the monitoring efforts for the determination of the levels of these compounds in the environment are extremely limited for several reasons. The primary reasons are the nonavailability of standardized sampling methods and the specialized analytical techniques that must be used for the determination of traces of these difficult to separate compounds in

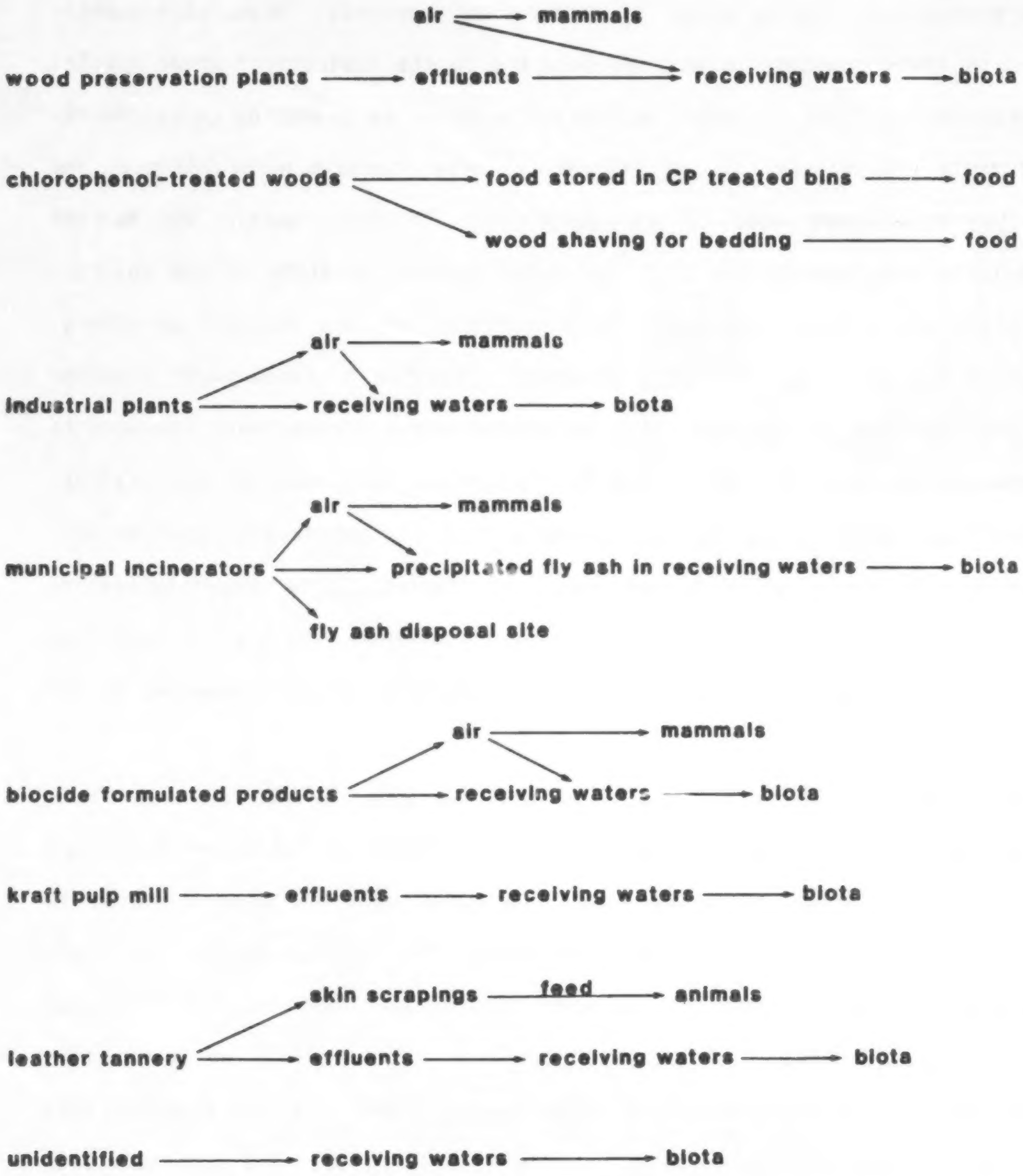


FIGURE 4-2

Possible Potential Relationship Between Various Sources of PCDDs and the Environmental Matrices Where PCDDs have been Detected

Source: Modified from NRCC, 1981a

the presence of a large number of interfering compounds. Measurable quantities of these compounds have been detected in the environment under special circumstances, that is, after accidents in factories producing chlorophenols and their derivatives, in the environment after certain herbicide use, and in the environment near certain dumpsites. In other words, the current available data demonstrate that the major sources of PCDDs in the environment are those associated with the production, use and disposal of chlorophenols and their derivatives. Choudhary (1983) in a review paper provided a list for some of the potential workplaces where occupational exposure to PCDDs may occur. It should also be recognized that most of the environmental monitoring investigations measured 2,3,7,8-TCDD levels, whereas monitoring data for other PCDDs are even more limited. With these limitations in mind, the levels of 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD in various environmental media have been presented in the following subsections.

4.5.1. **Water.** NAS (1977) reported that no 2,3,7,8-TCDD has ever been detected in drinking water using methods with limits of detection in the ppt range. Other PCDDs including PeCDD and HxCDD have not been detected in drinking water. However, TCDD, including the 2,3,7,8-isomer, has been reported in aqueous industrial effluent samples and leachates from hazardous waste disposal sites. For example, Van Ness et al. (1980) analyzed eight effluents from a trichlorophenol manufacturing plant site and detected TCDD in two of these effluents (detection limit 10-30 pg/g). The concentrations of TCDD in the two samples with detectable TCDD concentrations were 17 and 100 pg/g. Although the specific isomer was not routinely separated, the authors concluded from their study that a significant portion of the TCDD was apparently the 2,3,7,8-isomer.

The analysis of leachate samples from two waste disposal sites for the analysis of TCDD have also been reported. In one study, 23 water samples analyzed by Wright State University inside and outside of a waste disposal site near Jacksonville, AR (containing wastes from 2,4-D and 2,4,5-T manufacture) were found to contain 2,3,7,8-TCDD (Thibodeaux, 1983). The concentration of 2,3,7,8-TCDD in these samples averaged 14 ppt with a concentration range of none detected to 47 ppb. In another study (U.S. EPA, 1982b), two untreated leachate samples collected from the Love Canal, NY, chemical dump site showed a concentration of 1.56 ppb (1560 ppt) for 2,3,7,8-TCDD. The treated leachate (samples taken after remedial steps were installed to minimize PCDD-leaching possibility), on the other hand, showed no detectable level of 2,3,7,8-TCDD (detection limit 5-10 ppt). 2,3,7,8-TCDD was not detected in any of the groundwater samples analyzed.

Shadoff et al. (1977) analyzed for 2,3,7,8-TCDD in two locations exposed annually to 2,4,5-T. These locations were an impoundment from the drainage of a watershed in Texas where 2,4,5-T had been used for several years for brush control and from a pond in Arkansas used as a reservoir for irrigating rice fields treated with 2,4,5-T. Two water samples from each location failed to show any detectable level of 2,3,7,8-TCDD at a detection limit of 0.1-0.2 ppt.

4.5.2. Air. One possible source of PCDDs in the atmosphere is the field spraying of the herbicide 2,4,5-T. The spraying of 2,4,5-T containing 2,3,7,8-TCDD impurity may lead to a concomitant exposure to 2,3,7,8-TCDD. However, the measurement of air concentration at any particular time after spraying may not be a representative sample because of spray drift to non-target sites and the intermittent nature of spray application. From micro-agroecosystem chamber and field studies, Nash and Beall (1980) determined the atmospheric concentration of 2,3,7,8-TCDD at various times after the application of emulsified and granular Silvex (1.3-2.0 kg/ha Silvex) containing 44 ppb to 15 ppm TCDD impurity. Using tritiated 2,3,7,8-TCDD, these authors found that atmospheric concentrations of 2,3,7,8-TCDD decreased with time either at an exponential rate (granular formulation) or at a log log rate (emulsifiable formulation) in chambers. The emulsifiable formulation resulted in considerably higher TCDD concentrations (~1000-fold or more) in air than in granular formulation initially, but with time (200 days) approached the concentrations in air similar to the granular formulation (10 fg/m$^3$; fg $\equiv$ $10^{-15}$ g). In a small field trial, with a nonshaded plot, TCDD concentrations in air from the application of 2 kg/ha of emulsifiable Silvex containing 15 ppm TCDD were about twice (620 fg/m$^3$) that of a shaded plot (270 fg/m$^3$) on the treatment day, but only ~33% of the amount from the shaded plot on the second day. Presumably, this was a result of the lesser quantities (<50%) of TCDD remaining on the grass for volatilization during the second day.

Air filter samples collected from Elizabeth, NJ, after an industrial fire on April 22, 1980, were analyzed for TCDD by Harvan et al. (1981). Collision-induced-dissociation mass-analyzed ion kinetic energy spectrometry was used for the confirmation of the presence of TCDD. Of the nine samples analyzed by these authors, one contained 20 pg of TCDD, four contained <9 pg of TCDD, and four others probably contained 5-12 pg of TCDD. However, the concentration of TCDD in the air cannot be given for these samples because the air volumes corresponding to the filters analyzed were not specified by the investigators.

The atmospheric concentrations of TCDD near two hazardous waste sites have been monitored. In one study, U.S. EPA (1982b) failed to detect (detection limit 1-20 ppt) any 2,3,7,8-TCDD in the atmosphere at the Love Canal, NY, area. In another study of a waste disposal site near Jacksonville, AR, an average concentration of 1100 ppt of TCDD in two air particulate samples collected near the disposal site was reported (Thibodeaux, 1983).

The levels of 2,3,7,8-TCDD in atmospheric dust were monitored in the Seveso, Italy, area between 1977 and 1979. The concentrations of 2,3,7,8-TCDD were found to be in the range of 0.06-2.1 ng/g of dust with dustfall jars as sample collection technique and 0.17-0.50 ng/g of dust with high volume sampler as sample collection technique (DiDomenico et al., 1980b). The accident in Seveso released only 2,3,7,8-TCDD, while most other environmental sources may produce a mixture of PCDDs.

Another source of atmospheric emission of PCDDs is incineration (Gizzi et al., 1982; Benfenati et al., 1983; Taylor et al., 1983; Olie et al., 1982, 1983; Lustenhouwer et al., 1980; Barnes, 1983). The concentrations of TCDD, PeCDD and HxCDD in fly ash from Canadian municipal incinerators have been studied extensively by Eiceman et al. (1980, 1981). Eiceman et al. (1979) also determined the TCDD levels in fly ash from incinerators in Japan and the Netherlands. The average concentrations of the PCDDs in the Canadian studies (Eiceman et al., 1979, 1980, 1981) were estimated with the assumption that the SIM response factors for all the PCDDs were the same as the response factor from 1,2,3,4-TCDD used as a standard. However, the analytical method used by these authors has been criticized by Nestrick et al. (1982). Recently, Karasek et al. (1982) also determined the total TCDD, PeCDD and HxCDD levels in a French municipal incinerator to be none detected, 7.8 and 21.8 ng/g, respectively. It was also concluded by these authors that the PCDDs tend to concentrate in particles of lower mean size (30 μm vs. >850 μm).

In another study, Bumb et al. (1980) studied the PCDD level in fly ash from a municipal incinerator in Nashville, TN, several European municipal incinerators, and the industrial incinerators of the Dow Chemical Co. facility in Midland, MI. The TCDD concentrations were determined to be 7.7 ng/g (0.4 ng/g of 2,3,7,8-TCDD), 2-20 ng/g and 0-38 ng/g (2,3,7,8-TCDD not detected), respectively. The corresponding values of HxCDD were reported to be 14, 30-200 and 1-20 ng/g. However, the analytical method used by these investigators has been criticized by other investigators (Hay, 1979). Buser and Rappe (1983) and Buser and Bosshardt (1978) also analyzed the fly ash from incinerators in Switzerland and Canada. In one such study (Buser and Bosshardt, 1978), the total amount of PCDDs in the fly ash from a Swiss municipal and industrial incinerator were found to be 0.2 and 0.6 ppm, respectively. The dioxin isomers known to be most toxic, namely 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD, were only minor constituents of the total dioxins found. In another study (Buser and Rappe, 1983), the presence of TCDDs (3 ppb), PeCDDs (20 ppb) and HxCDDs (50 ppb) was indicated in the fly ash from a municipal incinerator in Zurich, Switzerland. The TCDD, PeCDD and HxCDD isomers with substitution at 2,3,7,8- positions, such as 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD and 1,2,3,6,7,8-, 1,2,3,7,8,9- and 1,2,3,4,7,8-HxCDD were present only as 2, 14 and 24% of the total TCDDs, PeCDDs and HxCDDs. A fly ash sample from Ontario, Canada, was also found to contain TCDDs (150 ppb), PeCDDs (550 ppb) and HxCDDs (900 ppb). Although the sample was reported to contain significantly higher levels of PCDDs in comparison with the Swiss fly ash sample, it showed similar proportions of 2,3,7,8-substituted PCDDs (4, 12 and 27% of the total TCDDs, PeCDDs and HxCDDs, respectively). It is not yet known whether the higher levels of PCDDs result from different incinerator operating conditions, different feed stock or different fly ash collection conditions (Buser and Rappe, 1983). Similarly, the fly ash from a municipal incinerator in the United States showed the presence of at least 11 TCDD isomers, but 2,3,7,8-TCDD was found to be a minor product (U.S. EPA, 1980a).

The U.S. EPA evaluated the magnitude and significance of TCDD emissions from combustion processes. In 1981, the U.S. EPA sampled five municipal waste combustors and concluded that emissions from these waste combustors do not present a public health hazard for residents living in the immediate vicinity (CEQ, 1981). In view of the recent data of Pocchiari et al. (1983) reporting the presence of 1,3,6,8- and 1,3,7,9-TCDD (0.4-2 ppt) in epigeal parts of a large number of plants grown in the proximity of municipal incinerators, and the toxicological evaluation of TCDD in ashes from urban incinerators (Bronzetti et al., 1983; Rizzardini et al., 1983), the question of health hazard for residents living in the immediate vicinity of municipal incinerators needs further evaluation. Another reason for the presence of 1,3,6,8- and 1,3,7,9-TCDD in epigeal parts of plants may also be due to contamination by TCDD-containing herbicide or pesticide application, as observed by Yamagishi et al. (1981).

4.5.3. Soil. The levels of PCDDs in soil, sediment and dust samples are presented in this subsection. In general, the PCDDs have been detected in the samples that originated from the areas around certain industrial sites, waste disposal sites, and sites involved in accidental or unintentional spillage of chemicals containing PCDD contaminants. Very few investigators determined the levels of other PCDDs besides TCDD. Even in the case of TCDD, the specific isomer identification was not performed in many cases. The levels of TCDD in different soil, sediment and dust samples are shown in Table 4-3.

TABLE 4-3

Levels of TCDD in Soils and Sediments from Different Locations

| Sample Type | Sampling Site | Sample History | Concentration in Sample |  | Reference |
|---|---|---|---|---|---|
|  |  |  | Total TCDD | 2,3,7,8-TCDD |  |
| Soils | Love Canal, NY | waste disposal site | <0.0025-6.7 ppb | NR | Smith et al., 1983b |
| Sediments | Love Canal, NY | sediments from storm sewers and creeks near water disposal site | NR | 0.9-312 ppb | Smith et al., 1983b |
| Soils | Love Canal, NY | soils collected away from source of contamination | NR | ND (1-20 ppt)[a] | U.S. EPA, 1982b |
| Sediments | Love Canal, NY | sediments from storm sewers | NR | ND (1-20 ppt)[a]-672 ppb | U.S. EPA, 1982b |
| Sediments | Love Canal, NY | sediments from sump | NR | ND (1-20 ppt)[a]-9570 ppb | U.S. EPA, 1982b |
| Soils | NR | sample originated from an industrial site | ND (20-2300 ppt)[a]-559 ppb | NR | Van Ness et al., 1980 |
| Soils | Eastern Missouri, U.S.A. | sample originated from contaminated horse arena | NR | detected[b] | Buser and Rappe, 1980 |
| Soils | Seveso, Italy | sample originated from ICMESA plant accident site | NR | detected[b] | Buser and Rappe, 1980 |
| Sediments | canal north of Amsterdam | sample originated from a dump site | NR | 55-5062 ppt | Heida, 1983 |
| Soils | Seveso, Italy | sample originated from ICEMSA plant accident site | NR | <5-20,000 µg/m$^2$ | DiDomenico et al., 1980c |
| Soils | Jacksonville, AR | waste disposal site | NR | ND-2.9 ppb | Thibodeaux, 1983 |

TABLE 4-3 (cont.)

| Sample Type | Sampling Site | Sample History | Concentration in Sample | | Reference |
|---|---|---|---|---|---|
| | | | Total TCDD | 2,3,7,8-TCDD | |
| Sediments | Jacksonville, AR | sediments from pond and creek near waste disposal site | NR | ND-22.1 ppb | Thibodeaux, 1983 |
| Soil/sludge | Love Canal, NY | waste disposal site | 0.3-199 ppb | NR | Tiernan, 1982 |
| Soils | unspecified Midwestern community in U.S.A | sample near a wire reclamation incinerator | ND (<3 ppt)[a]-<br>0.021 ppb | NR | Hryhorczuk et al., 1981 |
| Soil/dust | Midland, MI | sample inside industrial site | 1-120[c] ppb<br>1-4[d] ppb | 0.3-100[c] ppb<br>0.7-3[d] ppb | Bumb et al., 1980 |
| Soil/dust | Urban U.S. areas | no obvious source of contamination | ND (1-10 ppt)[a]-<br>0.03 ppb[c]<br>ND (1-10 ppt)[a]-<br>0.04 ppb[d] | NR[e] | Bumb et al., 1980 |
| Soils | Northwest Florida | Eglin Air Force test site | 0.010-0.70 ppb[f]<br>12.3 ppb[g] | NR | Cockerham et al., 1980 |
| Soils | Eastern Missouri | horse breeding arena sprayed with waste oil | 31.8-33.0 ppm | NR | Carter et al., 1975 |

[a] Not detected and the detection limit indicated within parentheses

[b] Value not quantified

[c] Value for soil

[d] Value for dust

[e] Dust sample from St. Louis, MO, area showed 0.12 ppb 2,3,7,8-TCDD.

[f] This is the soil residue after 10 years of periodic aerial spraying of 2,4-D and 2,4,5-T.

[g] This is the soil residue immediately after spraying.

NR = Not reported; ND = Not detected

It is obvious from Table 4-3 that the waste disposal site is responsible for the origin of 2,3,7,8-TCDD in the Love Canal, NY, area. This is reflected by the high level of 2,3,7,8-TCDD found in sediments from sump and in sediments from storm sewers and creeks near waste disposal sites. The reported levels of 2,3,7,8-TCDD in soil and sediment samples near the Jacksonville, AR, waste disposal site are such that this site requires careful reexamination. It can also be concluded from Table 4-3 that the environment inside a manufacturing (2,4,5-trichlorophenols and derivatives) site are likely to be contaminated with 2,3,7,8-TCDD by levels that may be higher than the background level (sites with no obvious sources of contamination).

4.5.4. Foods and Biological Samples. The occurrence of PCDDs in foods could result from the following: 1) spraying of certain grain crops with PCDD-contaminated herbicides, such as Silvex and 2,4,5-T; 2) consumption by livestock of PCDD-contaminated forage; 3) magnification of residues through the food chain; or 4) consumption of fruits and vegetables in the proximity of municipal incinerators. Besides determining the PCDD levels in food chains, this subsection will discuss the levels of these compounds in wildlife and in human tissues (i.e., urine and milk). The detection of these compounds in wildlife and human tissue collected near industrial or waste disposal sites can be taken as an indication of anthropogenic exposure. Sometimes the tissue levels can be used to estimate the extent of exposure and subsequent excretion and/or accumulation of these compounds.

The detection of 2,3,7,8-TCDD has been reported in locally grown garden fruit and vegetables following the ICMESA accident in Seveso, Italy, in 1976 (Fanelli et al., 1982; Cocucci et al., 1979; Pocchiari et al., 1983; Wipf et al., 1982). Studies with either the seeds or the mature plants of soybeans or oats showed that 2,3,7,8-TCDD was neither absorbed by the seeds after spraying nor taken up from the soil into the mature plants (Isensee and Jones, 1971; Matsumura and Benezet, 1973). However, young plants accumulated up to 40 ppb of 2,3,7,8-TCDD (Isensee and Jones, 1971). From the analysis of several parts of fruit trees and kitchen-garden plants such as carrots, onions, potatoes and narcissuses collected from the contaminated (400-1000 $\mu g/m^2$ of 2,3,7,8-TCDD in soil) Seveso area in Italy, Cocucci et al. (1979) concluded that 2,3,7,8-TCDD is translocated from soil to the aerial parts of the plants, probably through the conductive vessels. This study further suggested that the plants may eliminate 2,3,7,8-TCDD by an unknown mechanism within 4-10 months after transplantation in unpolluted soils. However, the study of Cocucci et al. (1979) contradicts the investigations of Wipf et al. (1982) in which vegetation samples analyzed from the Seveso area from 1976 through 1979 suggested that the contamination in vegetation was from local dust and not from plant uptake. Unlike the Seveso incident where release of 2,3,7,8-TCDD in the environment took place, normal use of herbicides containing 2,3,7,8-TCDD impurities may not cause detectable 2,3,7,8-TCDD contamination of the crop. Jensen et al. (1983) analyzed rice grain from fields in Arkansas, Louisiana and Texas after application of 2,4,5-T (containing 0.4 ppm TCDD) at a maximum rate of 2.25 pounds/acre. No 2,3,7,8-TCDD residues (detection limit 2-10 ppt) were found in these rice grains nor were any TCDDs found in 30 samples of rice purchased in retail stores throughout the United States. Contamination of fruits, vegetables or grains in the United States with TCDD has never been reported.

The contamination of a large number of vegetables grown in the proximity of municipal incinerators has been reported by Pocchiari et al. (1983). These investigators detected 1,3,6,8- and 1,3,7,9-TCDD in the concentration range of 0.4-2 ppt in vegetables whose origin of TCDD was not attributable to the ICMESA plant accident. This finding suggests the possibility of human exposure of TCDD from edible vegetables grown in areas close to municipal incinerators.

Different investigators have reported the presence of PCDDs in the fat of cattle that had grazed on pasture experimentally treated with 2,4,5-T (Meselson et al., 1978; Kocher et al., 1978). The levels of TCDDs in these studies ranged from 3-70 ppt. Kocher et al. (1978) reported that only 13% of the fat samples collected (3 of 23 samples) gave a positive response for 2,3,7,8-TCDD at low levels (3-4 ppt).

Results of a collaborative program to analyze a selected beef sample by the U.S. EPA, Dow Chemical Company, Wright State University and Harvard University showed that TCDD could be detected in the adipose tissue of cattle with access to 2,4,5-T-treated rangeland (U.S. EPA, 1984). Of the 85 beef fat samples analyzed, one sample contained 60 ppt of 2,3,7,8-TCDD and two samples appeared to have 2,3,7,8-TCDD levels in the range of 5-10 ppt. No 2,3,7,8-TCDD was determined in the rest of the samples. While several laboratories detected levels in this lower range, the values reported were very near the limits of detection.

Bovine milk collected after the accident in Seveso area was analyzed by Fanelli et al. (1980b). The concentration of 2,3,7,8-TCDD was found to vary from none detected (detection limit <40 ppt) to as high as 7.9 ppb. Other investigators have failed to detect either 2,3,7,8-TCDD (detection limit 1 ppt) or HxCDD (detection limit 25 ppt) in surveillance (after normal application of 2,4,5-T on pasture) samples of milk from the states of Oklahoma, Arkansas and Missouri, or quarantined milk in the state of Michigan (Lamparski et al., 1978; Mahle et al., 1977).

TCDDs including 2,3,7,8-TCDD, PeCDDs including 1,2,3,7,8-PeCDD and HxCDDs including 1,2,3,6,7,8-HxCDD have been detected in fish from a few PCDD-contaminated areas. This is discussed in detail in Section 6.2.

PCDDs have been detected in gelatin samples obtained from supermarkets and in bulk gelatin (Firestone et al., 1979). Eleven of 15 commercial gelatins examined contained a combined amount of 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD ranging from 30-700 ppt. Three bulk gelatins of Mexican manufacture showed higher levels of PCDDs. 2,3,7,8-TCDD was not detected in any sample. The origin of PCDDs in gelatin was speculated to be PCP and trichlorophenol that are routinely used in the leather-tanning industry. The use of by-product fat materials from PCP-treated hides as animal feed constituents led to widespread outbreaks of chick edema disease in the late 1950s (Firestone, 1973).

Bumb et al. (1980) analyzed charcoal-broiled steak under conditions representing rare, well-done and overdone samples and failed to detect either TCDD (detection limit 1-10 ppt) or HxCDD (detection limit 10-50 ppt) in the cooked meat of selected meat samples.

The analysis of human milk and urine for 2,3,7,8-TCDD has been performed. A study of 103 samples of breast milk from mothers living in areas within the United States that were sprayed with 2,4,5-T/silvex revealed no TCDD at a detection limit of 1-4 ppt and 0.1-10 ppt (U.S. EPA, 1980a; Heath et al., 1985). The control samples that were also negative for 2,3,7,8-TCDD were derived from mothers living in areas where no records for 2,4,5-T or silvex exposure exist (Heath et al., 1985).

In an interlaboratory collaborative analytical study on adipose tissue it was revealed that tissues from three Vietnam veterans heavily exposed to

Agent Orange contained 2,3,7,8-TCDD residues with levels ranging from 20-173 ppt (Gross et al., 1984). A survey of 72 autopsy tissue materials from across Canada found 2,3,7,8-TCDD averaging between 5 and 10 ppt, OCDD averaging between 600 and 800 ppt, and intermediate levels for penta-, hexa- and hepta-dioxins (Ryan et al., 1985). Results on the distribution of tetra- and octa-chlorinated dioxins in autopsy tissues from the general population, supported by the above observations, indicate that there is substantial contamination of the general population in the United States and Canada with 2,3,7,8-chlorine substituted tetra- through octa-dioxins (Schecter et al., 1985). Rappe et al. (1984, 1985) also reports the presence of PCDDs in human adipose tissue. In the same study Rappe et al. (1985) reported their analytical results on a survey of mothers' milk from Sweden, Germany, Denmark and Vietnam. All the samples contained different levels of PeCDD, HxCDD, HpCDD and OCDD residues. The most toxic isomer, 2,3,7,8-TCDD, was found only in the milk samples of mothers from Sweden and Germany but not Vietnam. This isomer was not analyzed in milk samples of mothers from Denmark (Rappe et al., 1985).

The monitoring of urine samples from two people involved with spray application (2,4,5-T) showed no detectable level of TCDD at a detection limit of ~2 ppt (Lavy et al., 1980).

## 4.6. EXPOSURE

The exposure of the general United States population to the four PCDDs cannot be estimated because the levels of these compounds in air, drinking water and foods have not been established. In fact, no PCDD contamination of any United States drinking water has ever been reported. Although the local atmosphere near a few chemical disposal sites and municipal incinerators has been reported to be contaminated with TCDD and HxCDD, no comprehensive study is available to demonstrate the atmospheric levels of these compounds in areas farther away from the point sources. Similarly, some of these compounds have been detected in edible aquatic species. Again, these fish contaminations have been reported in areas near a limited source where effluents contaminated with these compounds may have been discharged into surface waters. One of the consumer products that has been found to be contaminated with HxCDD is gelatin. However, it is difficult to estimate the contribution of food to human exposure of PCDDs from such limited data. It seems more prudent to try to estimate the exposure of these compounds to populations in certain localized areas (e.g., dump sites and known sources of industrial pollution) and certain special population groups (i.e., occupational) when adequate data are available.

The concentrations of 2,3,7,8-TCDD in bottom sediments of a drainage canal passing through a dump area (wastes from 2,4,5-T production) in northern Amsterdam, Holland, were reported by Heida (1983). The concentrations of 2,3,7,8-TCDD in sediments within the dump area varied from 844-5062 ppt and outside the dump area from 55-611 ppt. Analysis of the eel revealed that only two samples originating from shallow ponds adjacent to the main drainage canal contained between 1.0 and 1.1 ppt of 2,3,7,8-TCDD. 2,3,7,8-TCDD was not detected in other eel samples collected farther away from the dump site. This study demonstrates the possibility of TCDD contamination near dump sites.

The results of analysis for 2,3,7,8-TCDD and HxCDD in human milk samples were reported by Langhorst and Shadoff (1980). About 6 of the 9 samples showed 2,3,7,8-TCDD at levels slightly higher than the detection limits (0.2-0.7 ppt). All nine samples showed HxCDD at levels slightly higher than the detection limit (0.2-0.5 ppt). However, these results remain unconfirmed because of the lack of validation of the precision and accuracy of data. Investigations of 103 breast milk samples from mothers living in areas in the United States sprayed with 2,4,5-T revealed no TCDD at a detection limit of 1-4 ppt (U.S. EPA, 1980a).

The monitoring of urine samples from two people involved with spray application (2,4,5-T) showed no detectable level of TCDD at a detection limit of ~2 ppt (Lavy et al., 1980).

In one Polish study (Gorski, 1981), 1,2,3,6,7,8-HxCDD was detected in latex nipples at a concentration of 20-400 ppt. However, no TCDD or PeCDD was detected. The origin of PCDDs in the latex was speculated to be the result of γ-irradiation of latex (for crosslinking) containing PCP during its manufacturing process.

A BCF relates the concentration of a chemical in aquatic species to the concentration in water. The steady-state BCFs for a lipid-soluble compound in the tissues of various aquatic species seem to be proportional to the percent lipid in the tissue. Thus, the per capita ingestion of a lipid-soluble chemical can be estimated from the per capita consumption of fish and shellfish, and a steady-state BCF for the chemical.

Data from a recent survey on fish and shellfish consumption in the United States were analyzed by SRI International (U.S. EPA, 1980b). These data were used to estimate that the per capita consumption of freshwater and estuarine fish and shellfish in the United States is 6.5 g/day (Stephan, 1980). In addition, this information was used with data on the fat content of the edible portion of the same species to estimate that the weighted average percent lipids for consumed freshwater and estuarine fish and shellfish is 3.0%.

Several equations have been developed for predicting the steady-state BCF for an organic compound from its octanol-water partition coefficient (Kenaga and Goring, 1980; Veith et al., 1980; Veith and Kosian, 1983). All of these depend on the availability of a useful value for the partition coefficient. Several estimated values (Leo, 1979; Mabey et al., 1981; Neely, 1983) and one measured value (Neely, 1979; Kenaga, 1980; Neely, 1983) have been reported for the octanol-water partition coefficient for 2,3,7,8-TCDD. Use of six equations with four values for the partition coefficient, $K_{ow}$, results in the following predicted BCFs (Table 4-4). The predicted BCFs range from 7000-900,000 using the calculated values of the partition coefficient and from 3000-68,000 using the one measured value.

Several measured BCFs have been reported for 2,3,7,8-TCDD (Table 4-5), but none can be considered definitive values. Many were determined in model ecosystems in which the concentrations in water were not necessarily constant. The measured BCFs, however, range from 2000-9000. A few other BCF values are given in Table 5-1. Until further information is available, the U.S. EPA's best current estimate for the BCF of 2,3,7,8-TCDD in aquatic organisms is 5000. An adjustment factor of 3.0/7.6=0.39 can be used to adjust the estimated BCF from the 7.6% lipids on which the equation is based to the 3.0% lipids that is the weighted average percent lipids consumed per capita from fish and shellfish (U.S. EPA, 1980b). The weighted average BCF for 2,3,7,8-TCDD in the edible portion of all freshwater and estuarine aquatic organisms consumed by Americans is calculated to be 5000x0.395=1975. Uptake by fish from lower tropic levels may add to uptake from water, so this BCF may underestimate concentrations in wild aquatic organisms.

## TABLE 4-4

### Predicted BCFs from Calculated and Measured Values of $K_{ow}$ [a]

| Equation | log $K_{ow}$ | | | |
|---|---|---|---|---|
| | Calculated | | | Measured[b] |
| | 6.84 | 7.14 | 7.28 | 6.15 |
| $\log BCF = 0.542 \log K_{ow} + 0.124$ | 6,780 | 9,860 | 11,700 | 2,870 |
| $\log BCF = 0.76 \log K_{ow} - 0.23$ | 93,000 | 157,000 | 201,000 | 27,800 |
| $\log BCF = 0.79 \log K_{ow} - 0.40$ | 101,000 | 174,000 | 224,000 | 28,740 |
| $\log BCF = 0.635 \log K_{ow} + 0.7285$ | 118,000 | 183,000 | 225,000 | 43,000 |
| $\log BCF = 0.85 \log K_{ow} - 0.70$ | 130,000 | 234,000 | 308,000 | 33,700 |
| $BCF = 0.048\ K_{ow}$ | 332,000 | 663,000 | 915,000 | 67,800 |

[a] Sources: Kenaga and Goring, 1980; Veith et al., 1980; Veith and Kosian, 1983

[b] This measured value has been reported by Neely, 1979

## TABLE 4-5

### Measured Bioaccumulation Factor for 2,3,7,8-TCDD in Freshwater Aquatic Organisms

| Species | Tissue | Percent Lipid | Duration (days) | Bioconcentration Factor | Reference |
|---|---|---|---|---|---|
| Alga,<br>*Oedogonium cardiacum* | NR | NR | 33 | 3094[a] | Isensee, 1978 |
| Alga,<br>*Oedogonium cardiacum* | NR | NR | 32 | 2075[b]<br>2083 | Isensee, 1978<br>Yockim et al., 1978 |
| Snail,<br>*Physa* sp. | whole body | NR | 33 | 5471[a] | Isensee, 1978 |
| Snail,<br>*Physa* sp. | whole body | NR | 32 | 2095[b]<br>3731 | Isensee, 1978<br>Yockim et al., 1978 |
| Cladoceran,<br>*Daphnia magna* | whole body | NR | 30 | 3895[a] | Isensee, 1978 |
| Cladoceran,<br>*Daphnia magna* | whole body | NR | 32 | 7070[b]<br>7125 | Isensee, 1978<br>Yockim et al., 1978 |
| Catfish,<br>*Ictalurus punctatus* | whole body | NR | 28 | 2000 | U.S. EPA, 1983a;<br>Thomas, 1983 |
| Mosquitofish,<br>*Gambusia affinis* | whole body | NR | 14 | 4850[b]<br>4875 | Isensee, 1978<br>Yockim et al., 1978 |
| -- | - | NR | - | 9080[c] | Neely, 1979 |
| -- | - | NR | - | 5400 | Kenaga, 1980 |

[a] These are arithmetic mean of several values given

[b] These are values at equilibrium tissue concentrations

[c] Calculated as ratio of uptake and clearance rate constants

NR = Not reported

The BCF for 2,3,7,8-TCDD in the earthworm, Allobophora caliginosa or rosea, from soil with initial 2,3,7,8-TCDD concentration in the range of 0.06-9.2 ppb has been determined to be ~ 10 (Fanelli et al., 1982).

The BCFs for other PCDDs cannot be estimated because of the lack of solubility data.

Finally, the levels of TCDD in wildlife have been determined by various authors and are discussed in detail in Section 6.2.

## 4.7. SUMMARY

None of the PCDDs are commercially manufactured in the United States or anywhere else in the world. They are produced as unwanted contaminants during the manufacture of primarily chlorophenols and their derivatives, such as the herbicides 2,4,5-T and Silvex. At the present time, there is no known manufacturer of trichlorophenol in the United States. Its derivatives distributed in the market before banning, however, continue to be used as pesticides in the United States. The level of 2,3,7,8-TCDD contaminants in commercially available 2,4,5-T and similar formulations had been reduced to <0.1 ppm before these products were banned.

The primary sources of PCDDs in the environment probably are industrial manufacturers of chlorophenols or their derivatives, and chemical disposal sites containing the wastes from these industries. Municipal waste incineration also may produce some environmental emission of PCDDs. The significance of this source of emission compared with industrial emission and probable contamination from chemical disposal sites cannot be assessed with the available data. The 1,2,3,7,8-PeCDD now found in environmental samples has only been reported in emissions from incinerators.

PCDDs, particularly TCDD and its specific isomer 2,3,7,8-TCDD, have been monitored in a number of environmental media, including air, water, soil, food and biological media. The monitoring data to date indicate that the maximum level of PCDDs is likely to be found in soil and drainage sediment samples near chlorophenol manufacturing industries and chemical waste disposal sites. PCDDs have rarely been monitored in United States air samples. Small amounts of PCDD contamination have been found in fish and wildlife in the United States in areas around chlorophenol manufacturing industries and chemical waste disposal sites.

# 5. ENVIRONMENTAL FATE AND TRANSPORT PROCESSES

## 5.1. FATE

### 5.1.1. Water.

5.1.1.1. BIODEGRADATION -- 2,3,7,8-TCDD exhibits relatively strong resistance to biodegradation. Only 5 of ~100 microbial strains that have the ability to degrade persistent pesticides show slight ability to degrade 2,3,7,8-TCDD (Matsumura and Benezet, 1973). Ward and Matsumura (1977) studied the biodegradation of $^{14}C$-labeled 2,3,7,8-TCDD by using lake waters and sediments from Wisconsin. The observed half-life of 2,3,7,8-TCDD in sediment-containing lake waters was found to be 550-590 days. In lake water alone, ~70% of the 2,3,7,8-TCDD remained after 589 days. Using an outdoor pond as a model aquatic ecosystem and dosing it with $^{14}C$-labeled 2,3,7,8-TCDD, Tsushimoto et al. (1982) and Matsumura et al. (1983) estimated the apparent half-life of 2,3,7,8-TCDD to be ~1 year. Although biodegradation may have been responsible for part of the degradation, it is almost impossible to estimate the biodegradation half-life of 2,3,7,8-TCDD in aquatic systems from this experiment. It is likely that the apparent biodegradation loss was due to volatilization through air/water interface. Other investigators (Huetter and Philippi, 1982; Camoni et al., 1983) have demonstrated the virtually complete lack of degradation of 2,3,7,8-TCDD by microorganisms. It could be inferred from these studies that PeCDD and HxCDD, having more chlorine substitution on benzene rings, would be even more resistant to biodegradation than 2,3,7,8-TCDD.

The biodegradation half-life of 2,3,7,8-TCDD can also be estimated from the theoretical rate constant values based on relative rates of transformation reported in the literature or on structure-activity analogy values given by Mabey et al. (1981). Assuming the estimated biotransformation rate constant of $1 \times 10^{-10}$ mℓ $\mathrm{cell}^{-1}$ $\mathrm{hour}^{-1}$ (Mabey et al., 1981) and the concentration of microorganisms capable of degrading TCDD as $5 \times 10^{5}$ cell $\mathrm{m\ell}^{-1}$ (Burns et al., 1981), the half-life of biodegradation can be estimated to be >1 year. It should be emphasized that the role that biodegradation plays in the removal of PCDDs from water is not clear.

5.1.1.2. PHOTOTRANSFORMATION -- 2,3,7,8-TCDD has a UV absorption maximum at 310 nm with an extinction coefficient of 5590 $\mathrm{M}^{-1}$ $\mathrm{cm}^{-1}$ (NRCC, 1981a). 2,3,7,8-TCDD in a pure state is photochemically stable but it will photolyze in sunlight in the presence of a hydrogen atom donating substrate (Crosby and Wong, 1977). For example, Plimmer et al. (1973) reported that a 2,3,7,8-TCDD suspension in distilled water remained unchanged when irradiated with a sunlamp. Similarly, a thin dry film of 2,3,7,8-TCDD on a glass plate or 2,3,7,8-TCDD on dry and wet soils showed negligible photodegradation after irradiation with sunlamps (Crosby et al., 1971). In contrast, 2,3,7,8-TCDD in methanol solution or benzene solution of 2,3,7,8-TCDD in water stabilized by surfactant underwent substantial photodegradation under sunlamp or sunlight irradiation (Plimmer et al., 1973; Crosby et al., 1971). Botre et al. (1978) demonstrated that cationic surfactants, namely 1-hexadecylpyridinium chloride, act as an energy transfer agent in facilitating the photodecomposition of TCDD in aqueous solutions. These laboratory studies may not be applicable to the ambient environments. To explain the longer half-life of 2,3,7,8-TCDD in a model laboratory ecosystem than in an outdoor pond, Matsumura et al. (1983) and Tsushimoto et al. (1982) speculated that photolysis was the most likely cause. In the outdoor environment where the intensity of sunlight was higher compared with the laboratory experiments, algae-mediated photosensitization of 2,3,7,8-TCDD may cause some photodecomposition of this compound. Nestrick et al. (1980) estimated the photolytic half-life of 2,3,7,8-TCDD in n-hexadecane under sunlamp irradiation to be ~57 minutes. From the available information, it is difficult to predict the fate of 2,3,7,8-TCDD in aquatic media under environmental photolytic conditions. In the presence of hydrogen atom donating substrate(s) in surface waters, photolysis may be a significant fate process.

An increase in chlorine substitution is expected to decrease the rate of photodegradation (Nestrick et al., 1980; Helling et al., 1973). For example, Crosby et al. (1971) showed that although complete decomposition of 2,3,7,8-TCDD in methanol occurred in 24 hours under UV irradiation, >80% OCDD in methanol remained unreacted during the same period under similar irradiation conditions.

Although the degree of photolysis may be related to the extent of chlorination, positional isomerization also plays a critical and perhaps dominant part in the photolysis of higher PCDDs. In higher PCDDs, there appears to be preferential loss of chlorine from the 2, 3, 7 and 8 positions (Nestrick et al., 1980; Buser and Rappe, 1978; Choudhry and Hutzinger, 1984). However, Buser (1979) observed the formation of 2,3,7,8-TCDD in trace quantities, and PeCDD form photolysis of 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD. PCDD compounds with chlorine substitutions in positions 2, 3, 7 and 8 are likely to photodegrade faster than compounds not having these positional substitutions. According to such a predicted rule, it is not likely that photodegradation of OCDD and other higher PCDDs will yield a high quantity of 2,3,7,8-TCDD as the stable end product. For example, the photolysis half-life of 1,2,3,7,8-PeCDD has been estimated to be 7.6 hours in n-hexadecane solution under sunlamp irradiation (Nestrick et al., 1980). Similarly, the photolytic half-lives of 1,2,3,7,8-PeCDD, 1,2,3,6,7,9-HxCDD and 1,2,4,6,7,9-HxCDD in hexane solutions under sunlight irradiation have been determined to be 5.4, 17 and 47 hours, respectively (Dobbs and Grant, 1979). Nestrick et al. (1980) reported a half-life value of 6.8 hours for 1,2,3,6,7,8-HxCDD in n-hexadecane under sunlamp irradiation. The intermediates of the photodegradation of higher PCDDs are probably lower chlorinated dioxins, but the pathways of degradation are not known with certainty (NRCC, 1981a).

From the preceding discussions of the photolysis of PCDDs in the presence of organic hydrogen donating substrates, it is difficult to predict the photolytic fate of these compounds in natural aquatic media where sufficient organic hydrogen donating substrate(s) may or may not be available. The situation is complicated further by the fact that, unlike in solution, a predominant amount of PCDDs in surface water may remain sorbed on suspended particles and settled sediments. Moreover, since the penetration of UV light into natural water may be very limited, photolytic degradation of PCDDs is not likely to be of environmental importance.

5.1.1.3. RADICAL OXIDATION AND HYDROLYSIS -- Although these processes occur, hydrolysis of 2,3,7,8-TCDD or oxidation with free radicals ($RO_2\cdot$, $RO\cdot$, etc.) in aquatic media are not likely to be of environmental significance (Callahan et al., 1979; Mabey et al., 1981). Likewise, hydrolysis and oxidation are even less likely to be environmentally significant processes for PeCDD and HxCDD.

5.1.1.4. VOLATILIZATION -- Although several investigators implicated volatilization as one of the major reasons for the observed disappearance of 2,3,7,8-TCDD from aqueous solution during microbial studies, no quantitative information regarding the volatilization of 2,3,7,8-TCDD from aquatic media is available (Ward and Matsumura, 1977; Matsumura et al., 1983; Huetter and Philippi, 1982). 2,3,7,8-TCDD may undergo some water-mediated evaporation in aquatic media (Matsumura et al., 1983). Using the formulas of Liss and Slater (1974), a vapor pressure value of $1.7\times10^{-6}$ torr (0.2 m Pa) and a solubility value of $6.2 \times 10^{-10}$ mole/ℓ, the volatilization half-life for 2,3,7,8-TCDD was 6 minutes from water of 1 cm depth and 10 hours from water of 1 m depth (NRCC, 1981a). The limitations of this theory to predict the rate of volatilization have been discussed in the NRCC (1981a) document. The Liss-Slater model does not consider terrestrial matrices (suspended solids, sediments, biota, etc.) normally encountered in natural surface water and thus ignores the effects of these parameters on the volatilization rate. Employing a computerized EXAMS model for two standardized aquatic ecosystems (lake and pond; see NRCC, 1981a, for definitions) and the input parameters for 2,3,7,8-TCDD given in NRCC (1981a), volatilization has been estimated to account for 100% of the fraction lost; biodegradation has been calculated to be 0%. The volatilization half-life for TCDD has been estimated to be 5.5 and 12 years from pond and lake water, respectively. A transport model has also been used to estimate the volatilization rate of 2,3,7,8-TCDD from a cooling pond on an industrial site (Thibodeaux, 1983). The model accounted for movement of 2,3,7,8-TCDD from the bottom sediment to the water column and then to the air. Based on the measured concentrations in the pond bottom sediment (22,100 ng/kg) and the pond surface area (15,050 $m^2$), the calculated volatilization rate was 15-16 mg/year.

Pertinent data regarding the volatilization of PeCDDs and HxCDDs from aquatic media could not be found in the available literature. However, these compounds with higher molecular weight and more chlorine substitution are expected to volatilize more slowly than 2,3,7,8-TCDD from aquatic media.

5.1.1.5. SORPTION -- Data from microcosm experiments indicate that 2,3,7,8-TCDD is highly sorbed to sediments and biota (Isensee and Jones, 1975; Ward and Matsumura, 1978). More than 90% of 2,3,7,8-TCDD in an aquatic medium may be present in the adsorbed state (Ward and Matsumura, 1978; Matsumura et al., 1983). Considering the low water solubility and the high octanol/water partition coefficient, this is not surprising. In fact, the equation of Karickhoff et al. (1979) predicts a sorption partition coefficient value of $10^4$ for 2,3,7,8-TCDD in sediments containing 2% organic carbon. Similarly, the higher PCDDs are likely to be present predominantly in the sediment-sorbed state in aquatic media.

5.1.2. Air. A number of PCDDs, including TCDDs, PeCDDs and HxCDDs, have been detected in the dust and fly ash from municipal incinerators (Cavallaro et al., 1980b; Clement and Karasek, 1982; Eiceman et al., 1981). Size fractionations of fly ash from municipal incinerators have shown that larger concentrations of 2,3,7,8-TCDD and PeCDDs occurred on the larger (550 μm) particles, while the 30 μm particles had greater relative concentrations of OCDD (Clement and Karasek, 1982). Tiernan et al. (1982b) also reported higher concentrations of TCDDs on larger particles (3-10 μm) from a refuse-fueled municipal incinerator effluent than on smaller particles (<1 μm). PCDDs emitted to the atmosphere from combustion processes appear to be associated with air particulate matter (Nestrick et al., 1980). Atmospheric PCDDs originating from other noncombustion sources, such as herbicide-treated soils and vaporized PCDDs from aquatic media (Thibodeaux, 1983), are also likely to be associated with air particulate matter. Cupitt (1980) presented mathematical descriptions of physical removal mechanisms for the fate of toxic and hazardous materials in the air environment. For the adsorption of chemicals on aerosol particles he developed a general model based on aerosol surface area and chemical saturation vapor pressure. His results suggest that adsorption will be a reasonable vapor-phase removal mechanism from air only for materials with saturation vapor pressures of $10^{-7}$ torr (mm) or less. 2,3,7,8-TCDD has an estimated vapor pressure of $1.7\times10^{-6}$ torr.

Photodegradation and wet and dry deposition of particulate-bound PCDDs are probably the most important fate-determining processes for the atmospheric PCDDs. The available data relating the photodegradation of these compounds in the sorbed phase or as films are conflicting. For example, experiments of earlier investigators involving photoreactivity of 2,3,7,8-TCDD as films or sorbed on solid surfaces and exposed to the atmosphere yielded negligible photodegradation with sunlight (Crosby et al., 1971). However, the more recent work of Buser (1979) and the investigations of the other researchers (Plimmer, 1978; Crosby and Wong, 1977) have shown that some photolysis of TCDD in the condensed phase (i.e., coated on glass plate or on silica) may take place. In the condensed phase, photodecomposition of TCDD in the bottom layers that are shielded from incident light by the surface layer is prevented.

Gebefuegi et al. (1977) studied 2,3,7,8-TCDD photochemical degradation under simulated environmental conditions by exposing silica gel-sorbed 2,3,7,8-TCDD to light of wavelength >290 nm and observed 92% decomposition in 7 days. The half-life for photodegradation of 2,3,7,8-TCDD film on glass surfaces has been estimated to be 5.8 days under irradiation with sunlamps (Nestrick et al., 1980). It is not known whether a similar photodegradation of particle-bound 2,3,7,8-TCDD will occur in the atmosphere since the state of sorption may be different from those obtained under laboratory conditions. The potential for oxidation of PCDDs by free radicals (OH•, O•, etc.) and other molecules ($O_3$, $NO_x$, etc.) that may be present in the atmosphere is unknown.

### 5.1.3. Soil.

5.1.3.1. SORPTION -- From the empirical correlation of Karickhoff et al. (1979), it is possible to predict a soil/water partition coefficient of $4.8x10^4$ for a soil containing 10% organic matter.

Because of their high affinity toward soils, particularly those with significant organic content, and because of their extremely low water solubilities, 2,3,7,8-TCDD (and presumably other PCDDs) tend to remain on or near the surface of soils (U.S. EPA, 1984). With time, 2,3,7,8-TCDD bound to soil becomes more difficult to desorb (Philippi et al., 1981; Huetter and Philippi, 1982).

Several authors have shown that vertical movement of 2,3,7,8-TCDD in soil is negligible, although movement of 2,3,7,8-TCDD may occur by horizontal transfer (eroded soil transported by water) and through contaminated airborne dust particles (U.S. EPA, 1984; Helling et al., 1973). Therefore, underground water supplies are unlikely to be contaminated with 2,3,7,8-TCDD. However, as the organic content of soil decreases, the likelihood of vertical movement of PCDDs in soil increases. In areas of heavy rainfall and sandy soil, vertical migration of 2,3,7,8-TCDD and its lateral displacement by soil erosion and runoff would be enhanced (U.S. EPA, 1984). The downward vertical migration of 2,3,7,8-TCDD up to 30 cm into soil has been suggested to have occurred in Seveso, Italy (DiDomenico et al., 1980d,e). The monitoring of Seveso soil 1 year after the accident showed that the highest 2,3,7,8-TCDD levels were not present in the topmost soil layer (0.5 cm), but very often in the second (0.5-1.0 cm) or third (1.0-1.5 cm) layers. In view of the low water solubility of 2,3,7,8-TCDD, probable explanations of this vertical distribution could be due to volatilization through the air/soil interface or solvation of 2,3,7,8-TCDD by organic solvents (NRCC, 1981a), or biotic mixing by earthworms or other soil invertebrates. It is, therefore, possible that 2,3,7,8-TCDD may appear in the air above and in normal water leachate of soils, particularly after multiple PCDD application or accidental release of 2,3,7,8-TCDD on soil.

5.1.3.2. **PHOTOTRANSFORMATION** -- The photodecomposition of 2,3,7,8-TCDD on wet or dry soil under artificial and natural sunlight was studied by Crosby et al. (1971). The photodecomposition was found to be negligible in soils. Similarly, Plimmer et al. (1973) determined that photodecomposition of TCDD on soils was too slow to be detected. In a later experiment, Plimmer (1978) found that although TCDD decomposed significantly from precoated silica plate (~22%) in 8 hours of sunlight irradiation, practically no decomposition of TCDD was observed from TCDD sorbed on soil under similar conditions.

The photodegradation of TCDD in combination with other pesticide mixtures was studied by Crosby and Wong (1977). When Agent Orange containing 15 ppm of TCDD was applied on the surface of glass plates (5 mg/cm$^2$), rubber plant, Hevea brasiliensis (6.7 mg/cm$^2$), and on the surface of sieved Sacramento loam soil (10 mg/cm$^2$) and exposed to sunlight, TCDD was found to photodecompose. The loss of TCDD in 6 hours was >50% from glass plate, ~100% from the surface of leaves and ~10% from the surface of soil. The rapid photolysis of TCDD from these surfaces indicates that the herbicide formulation provided a hydrogen donor that probably allowed the photolysis to occur. The authors attributed the slower photolysis of 2,3,7,8-TCDD in soil to a shading effect by lower layers of soil particles.

5.1.3.3. **BIODEGRADATION** -- Poiger and Schlatter (1980) noted that 2,3,7,8-TCDD absorbs strongly onto soil particles, thereby reducing its bioavailability. Young (1983) also noted that 2,3,7,8-TCDD is not likely to metabolize readily by soil microorganisms. It can be concluded from the following discussions that the biodegradation half-life in soil is likely to be >1 year.

The overall half-life of 2,3,7,8-TCDD in soil has been reported to be 1-3 years by Kearney et al. (1972). Studies performed by the U.S. Air Force (Young et al., 1976; IARC, 1977) suggested that soil bacteria may biodegrade TCDD. The half-life of this chemical in soils under relatively dry conditions (Utah test area) was found to be ~330 days and in more moist soils and under warm conditions (Florida test area) was found to be ~190 days. This is consistent with the biodegradation half-life of ~0.5 year for TCDD determined by Commoner and Scott from the soil in rural Missouri after the accidental spraying of TCDD-contaminated oil (IARC, 1977). However, these half-life estimates may greatly underestimate the true value, since it has recently been shown that radiolabeled TCDD adsorbed to soil becomes progressively more resistant to extraction (Philippi et al., 1981; Huetter and Philippi, 1982).

The rate of disappearance of 2,3,7,8-TCDD following an accidental 2,3,7,8-TCDD release from a trichlorophenol manufacturing plant at Seveso, Italy, was studied by DiDomenico et al. (1980d, 1982). The disappearance of 2,3,7,8-TCDD from the topmost soil layer after 1 year was speculated to be due to photodegradation, volatilization or vertical movement through the soil. These investigators estimated the initial half-life of 2,3,7,8-TCDD in soil at the time of its release to be 5 months. One month after release, the rate of disappearance of 2,3,7,8-TCDD slowed down to the equivalent of 1 year in apparent half-life. By the 17th month, the rate declined to an extremely slow level; the apparent half-life figure for this phase was calculated to be >10 years. More recent data (Young, 1983; Wipf and Schmid, 1983) indicate that the half-life of 2,3,7,8-TCDD in soil is about 10-12 years. Since most of the other PCDDs are no more susceptible to transformation/degradation than TCDDs, their half-lives in soil are presumed to be similar to that postulated for TCDDs.

5.1.4. **Food.** Isensee and Jones (1971) conducted experiments to study the possibility of absorption and translocation of 2,3,7,8-TCDD by plants from polluted soil. Oats and soybean plants grown to maturity in soil contaminated with 0.06 ppm 2,3,7,8-TCDD showed <1 ppb of 2,3,7,8-TCDD in the seeds. Cocucci et al. (1979) measured the level of contamination in kitchen garden plants (carrot, potato, onion and narcissus) grown in soil from the contaminated Seveso area containing 1000-4000 $\mu g/m^2$ of 2,3,7,8-TCDD. 2,3,7,8-TCDD was found to be 3-5 times higher in foliage than in fruits. The fact that the highest 2,3,7,8-TCDD content was found adjacent to the conductive tissue was interpreted as evidence of translocation of 2,3,7,8-TCDD from roots to the outer parts of the plants. The investigation of these authors also suggested that 2,3,7,8-TCDD may be eliminated from the mature plants. Wipf et al. (1982), however, failed to detect any measurable 2,3,7,8-TCDD in the flesh of fruits and vegetables collected from the contaminated area in Seveso during 1977-1979, although the soil 2,3,7,8-TCDD concentration was ~10 ppb. These authors concluded that 2,3,7,8-TCDD may not be translocated from soil to the plants. A similar conclusion was reached by Pocchiari et al. (1983) from their uptake experiments with plants. It can be concluded from these studies that 2,3,7,8-TCDD is not likely to concentrate in plants grown in contaminated soils.

With respect to potential 2,3,7,8-TCDD exposure through aerial parts of plants, when an aqueous suspension of pure 2,3,7,8-TCDD was exposed to either artificial light or sunlight, photodecomposition was negligible. However, in conjunction with other pesticides, 2,3,7,8-TCDD rapidly degraded when exposed to light (Crosby and Wong, 1977). This is consistent with the observations that TCDD was found not to persist on foliage (Sundstrom et al., 1979; Crosby and Wong, 1977) after application with other pesticides

(2,4,5-T, Agent Orange). The half-life of 2,3,7,8-TCDD disappearance from grass in Texas treated at a high rate (12 pounds/acre) of 2,4,5-T containing 0.4 ppm 2,3,7,8-TCDD was determined to be 5.6 days (Jensen et al., 1983). Cattle fed rations fortified with a maximum of 90 ppt TCDD were monitored for TCDD content in the body fat. TCDD from the body fat presumably disappeared with a half-life of ~16.5 weeks (Jensen et al., 1981). Similarly, cattle fed rations fortified with 500 ppt 2,3,7,8-TCDD showed a maximum level of 90 ppt of 2,3,7,8-TCDD in cows' milk. On withdrawal of 2,3,7,8-TCDD containing feed, 2,3,7,8-TCDD disappeared from the milk with a half-life of 41 days (Jensen and Hummel, 1982).

## 5.2. TRANSPORT

**5.2.1. Water.** The two likely transport processes for PCDDs in aquatic media are volatilization and sorption onto suspended particulates and subsequent sedimentation. No quantitative data regarding volatilization of any of these compounds from aquatic media are available, although several investigators implicated volatilization as one of the major reasons for the observed loss of 2,3,7,8-TCDD from aqueous solutions during microbial studies (Ward and Matsumura, 1977; Huetter and Philippi, 1982). There is a very wide difference in the calculated values of half-life of volatilization for 2,3,7,8-TCDD. For example, calculation based on the Liss and Slater (1974) equation gives a half-life for evaporation of 10 hours from water of 1 m depth (see Section 5.1.1.4.). Calculation based on a reaeration rate ratio of 0.373 (Mabey et al., 1981) and an oxygen reaeration rate constant of 0.19 $day^{-1}$, 0.96 $day^{-1}$ and 0.24 $day^{-1}$ (Mabey et al., 1981) for pond, river and lake water, respectively, gives half-life values of 10, 2 and 8 days for 2,3,7,8-TCDD in pond, river and lake water, respectively. These wide variations are conceivable when examined with the volatilization models for half-life (Thibodeaux, 1979). Evaporation half-life is shown to be proportional to water depth and inversely proportional to the mass-transfer coefficient. A more realistic calculation based on EXAMS predicts half-life values for TCDD of 5.5 and 12 years from pond and lake water, respectively (see Section 5.1.1.4.). The EXAMS calculation routine contains an added element that accounts for the sorption of TCDD both on the suspended and on-bottom sediment. For substances with high sorption coefficients such as TCDD, the evaporation rate is reduced significantly. A comparison of calculated transport rates from an industrial site indicates that evaporation of TCDD from a contaminated cooling water pond sediment is negligible in comparison with other contaminated areas on the site (Thibodeaux, 1983). It will also become apparent from the following discussion that volatilization may be insignificant compared with sorption processes for the transport of TCDD and presumably other PCDDs from aquatic media.

It has already been shown (see Section 5.1.1.5.) that 2,3,7,8-TCDD is highly sorbed to sediments and biota (Isensee and Jones, 1975) and >90% of 2,3,7,8-TCDD in aquatic media may be present in the sorbed state (Ward and Matsumura, 1978). This is consistent with the sorption partition coefficient value of this compound. Although the sorption effects of the higher PCDDs have not been studied, based on their expected higher octanol/water partition coefficient values, these compounds are likely to be present predominantly in the sediment-sorbed state in aquatic media.

5.2.2. Air. All the PCDDs are believed to be transported in the vapor-phase and in particulate bound form in the atmosphere (see Section 5.1.2.). The transport of these compounds from stationary point sources (i.e. stack emission) and area sources (waste disposal sites) can be theoretically predicted from dispersion modeling (Josephson, 1983). Although such dispersion modeling has been performed for 2,3,7,8-TCDD (SAI, 1980), the correlation between the theoretical value and experimental monitoring data has never been performed. In the case of accidental release of toxic clouds containing TCDD at Seveso, Italy, Cavallaro et al. (1982) determined the transport pattern and the ground deposition of the TCDD from the cloud. They determined that the TCDD deposition from air to soil should follow an exponential decay pattern along the downwind direction and follow a Gaussian-distribution along the cross-section of the downwind direction. From regression equations, these investigators determined that the aerial deposition $\gamma$ ($\mu g/m^2$) should be $\gamma = 2900\ e^{-2.3x}$ for $x<2$ km and $\gamma = 45\ e^{-0.5x}$ for 2 $km<x<6$ km. It is doubtful whether this equation can be used in the general case of accidental release of TCDD because of the varying meteorological conditions.

5.2.3. Soil. The probable media and modes of transport of PCDDs from soils are the following: 1) to air through contaminated airborne dust particles; 2) to surface water by eroded soil transported by water; 3) to groundwater by leaching; and 4) to air by volatilization. Movement of particulate matter containing sorbed PCDDs is considered to be a much more important transport mechanism than leaching because of the low water solubility of these compounds (Josephson, 1983). However, one year following the Seveso accident the highest 2,3,7,8-TCDD levels in soil were very often detected in the second (0.5-1.0 cm) or third (1.0-1.5 cm) layers but not in the top most soil layer (0.5 cm). This disappearance of at least a part of the 2,3,7,8-TCDD from the topmost soil layer was speculated to be due to volatilization or vertical movement down through the soil (DiDomenico et al., 1980d). Results of off-site transport calculations from contaminated soil surfaces are available (Thibodeaux, 1983). The calculations show that between 120 and 1200 g/year of TCDD were volatilized from a highly contaminated soil surface between 1978 and 1979 before the implementation of remedial measures. Over the same period it was estimated that 28-37 g/year left the site by wind-blown particle entrainment, 0.1-1.0 g/year evaporated from a burial site and 0.98-2.3 g/year in water runoff. All these sources are areas in which the 2,3,7,8-TCDD was found to remain sorbed on the soil. It appears that volatilization from soil and downward migration caused by soil movement, or through biotic mixing by earthworms or other soil invertebrates are more probable mechanisms by which 2,3,7,8-TCDD may be transported from soils.

## 5.3. BIOACCUMULATION/BIOCONCENTRATION

The bioconcentration of TCDD in various aquatic species has been studied under controlled laboratory conditions using static test chambers. The results of these investigations have been discussed in Section 4.6. and are given in Table 5-1. In all these experiments, the total amounts accumulated were found to be related to the initial TCDD concentrations in aquatic phase. The investigation of Philippi et al. (1981) made it clear that bioaccumulation would be significantly affected by the physical form (sorbed or in solution) in which TCDD occurs in the environment. Isensee (1978) reported that the concentration in the tissues of the tested species reached equilibrium in 7-15 days. In the absence of any experimental BCFs derived under dynamic test conditions, the values of Isensee (1978) reported in Table 5-1 probably represent the best experimental values available (in species other than fish) since these values were derived from equilibrium concentrations of TCDD in the tested tissues. The BCF for 2,3,7,8-TCDD in the earthworm, Allobophora caliginosa or rosea, from soil with initial 2,3,7,8-TCDD concentration in the range of 0.06-9.2 ppb has been determined to be ~10 (Fanelli et al., 1982).

## TABLE 5-1

### Bioconcentration Factor of TCDD for Several Aquatic Organisms[a]

| Species | Initial Aquatic Concentration (ppt) | Bioconcentration Factor | Reference |
|---|---|---|---|
| Algae, *Oedogonium cardiacum* | 0.05-1300 | 2,075 | Isensee, 1978 |
| Algae, *Oedogonium cardiacum* | 0.05-1300 | 9,000[b] | Isensee and Jones, 1975 |
| Algae, *Oedogonium cardiacum* | 0.1[c] | 2,080 | Yockim et al., 1978 |
| Ostracod | 2.6 | 110 | Matsumura and Benezet, 1973 |
| Duckweed, *Lemna minor* | 0.05-1300 | 3,625[b] | Isensee and Jones, 1975 |
| Snail, *Physa* sp. | 0.05-1300 | 2,095 | Isensee, 1978 |
| Snail, *Physa* sp. | 0.05-1300 | 20,000[b] | Isensee and Jones, 1975 |
| Snail, *Helosoma* sp. | 0.1[c] | 2,080 | Yockim et al., 1978 |
| Daphnids, *Daphnia magna* | 0.05-1300 | 7,070 | Isensee, 1978 |
| Daphnids, *Daphnia magna* | 0.05-1300 | 26,000[b] | Isensee and Jones, 1975 |
| Daphnids, *Daphnia magna* | 0.4 | 2,200 | Matsumura and Benezet, 1973 |

TABLE 5-1 (cont.)

| Species | Initial Aquatic Concentration (ppt) | Bioconcentration Factor | Reference |
|---|---|---|---|
| Mosquito fish, *Gambusia affinis* | 0.05-1300 | 4,850 | Isensee, 1978 |
| Mosquito fish, *Gambusia affinis* | 0.05-1300 | 26,000[b] | Isensee and Jones, 1975 |
| Mosquito fish, *Gambusia affinis* | 0.1[c] | 4,875 | Yockim et al., 1978 |
| Mosquito larvae, *Aedes aegypti* | 0.45 | 9,200 | Matsumura and Benezet, 1973 |
| Brine shrimp, *Artimia salina* | 0.1[c] | 1,570 | Matsumura and Benezet, 1973 |
| Catfish, *Ictalurus punctatus* | 0.05-1300 | 9,000[b] | Isensee and Jones, 1975 |
| Catfish, *Ictalurus punctatus* | 0.05-1300 | 4,875 | Yockim et al., 1978 |
| Brook Silverside, *Laludesthes sicculus* | 1.3 | 545[d] | Matsumura and Benezet, 1973 |
| Pond Weed, *Elodea nuttali* and *Ceratophyllon demersum* | 53.7 | 30,300 | Tsushimoto et al., 1982 |

[a] BCF values derived by Isensee and Jones (1975) were based on dry weight for all biological and sediment materials.

[b] Average of several values

[c] These are initial concentrations of TCDD in soil added to water.

[d] Error in the original publication corrected in the value reported here.

## 5.4. SUMMARY

The four transformation processes (photoreaction, biotransformation, hydrolysis and radical oxidation) that control the fate of a chemical in aquatic media do not appreciably transform TCDD and possibly other PCDDs in aquatic media. However, the two former processes may be more important for the transformation of 2,3,7,8-TCDD in aquatic media. The transport of these compounds to the atmosphere by volatilization from surface water may take place through a water-mediated process, particularly in the case of 2,3,7,8-TCDD, but significant transport of these compounds to the atmosphere through water may not be likely. Therefore, the PCDDs are expected to be very persistent in aquatic media.

The potential for oxidation of PCDDs by tropospheric free radicals is not known. Although appreciable photolysis of TCDD coated on glass plate or sorbed onto silica has been observed, it is not known whether a similar photodegradation of particle-bound TCDD and other PCDDs will occur in the atmosphere. The transport of vapor phase and particle-bound PCDDs may be theoretically predicted from dispersion modeling equations. In the case of accidental release of toxic clouds containing TCDD at Seveso, Italy, it has been demonstrated that the TCDD deposition from air to soil followed an exponential decay pattern along the downwind direction and a Gaussian distribution pattern along the cross-section of the downwind direction.

PCDDs are resistant toward photochemical and biodegradation reactions in soil. The half-life of 2,3,7,8-TCDD in soils may be >10 years. These compounds are likely to be transported from soil through movement of particulate matter containing sorbed PCDDs. The most probable transport mechanisms are transport of these compounds to the atmosphere by contaminated airborne dust particles, evaporation, and transport to surface water via eroded soil transported by water. Leaching is a less likely transport process for these chemicals except for very sandy soils.

Both the calculated and experimental results show that these compounds will bioaccumulate in aquatic organisms. The experimental BCF varies with the species and ranges from ~2000-30,000. However, studies with flow-through systems should be performed to establish the realistic bioaccumulation factors for these compounds in different aquatic species.

# 6. ECOLOGICAL EFFECTS

## 6.1. EFFECTS ON ORGANISMS

6.1.1. Aquatic Life Toxicology. Almost all of the available information concerning the toxicity of PCDDs to wildlife pertains to aquatic species, and most of the aquatic information is based on acute exposure to calculated, rather than measured concentrations of 2,3,7,8-TCDD.

6.1.1.1. ACUTE TOXICITY -- The effects of acute exposure to 2,3,7,8-TCDD have been reported for four species of freshwater fish and one species of amphibians (Table 6-1). In almost all of these studies, toxic effects were observed only after the acute exposure period ended. Miller et al. (1973, 1979) exposed juvenile coho salmon, *Oncorhynchus kisutch*, to a range of 2,3,7,8-TCDD concentrations for up to 96 hours. Concentrations were expressed as ng/g wet bw and as ng/ℓ of water, based on the amount of 2,3,7,8-TCDD added to the water in the test containers and the initial body weight of fish. Test concentrations were measured during the exposure period. After exposure, the fish were transferred to clean flowing water and observed for up to 114 days during which they were fed to satiation 3 times/week. Experiments were conducted with two groups of fish that differed in initial mean wet weight (3.51 and 6.63 g). Food consumption, growth and survival of smaller fish were measured until 60 days after exposure and were found to be significantly reduced at 5.4 μg/kg bw (0.0056 μg/ℓ), but not at 0.54 μg/kg bw (0.00056 μg/ℓ) or lower. Growth and survival of larger fish were measured until 114 days after exposure and were significantly reduced at 5.4 μg/kg bw (0.0105 μg/ℓ) but not at 0.54 μg/kg bw (0.00105 μg/ℓ) or lower. The actual concentrations in fish and water were undoubtedly lower than the calculated values, because much of the added 2,3,7,8-TCDD would be adsorbed to all containers.

TABLE 6-1

Effect of Acute Exposure to 2,3,7,8-TCDD on Aquatic Animals

| Species | Life Stage, Weight or Length | Duration of Exposure (hours) | Duration of Test (days) | $LC_{50}$ (μg/ℓ) | $LT_{50}$[a] (days) | Lowest Effect Concentration (μg/ℓ) | No Effect Concentration (μg/ℓ) | Effect | Reference |
|---|---|---|---|---|---|---|---|---|---|
| Coho Salmon, *Oncorhynchus kisutch* | 3.5 g | 96 | 64 | 0.0056 | 60 | 0.0056 | 0.00056 | reduced growth, food consumption, survival | Miller et al., 1973, 1979 |
| Coho Salmon, *Oncorhynchus kisutch* | 6.6 g | 96 | 114 | 0.0105[b] | 114 | 0.0105 | 0.00105 | reduced growth, food consumption, survival | Miller et al., 1973, 1979 |
| Rainbow Trout, *Salmo gairdneri* | eggs and larvae | 96 | 72 | NR | NR | 0.0001 | ND | temporary growth inhibition | Helder, 1981 |
| Rainbow Trout, *Salmo gairdneri* | eggs and larvae | 96 | 164 | NR | NR | 0.001 | 0.0001 | teratologic effects, decreased survival and growth | Helder, 1981 |
| Rainbow Trout, *Salmo gairdneri* | 0.85 g | 96 | 72 | NR | NR | 0.010 | NR | decreased survival and growth, histological effects | Helder, 1981 |
| Guppy, *Poecilia reticulata* | 9-40 mm | 120 | 37 | NR | 21.7 | 0.1 | ND | 100% mortality by 3.7 days after beginning exposure | Miller et al 1973; Norris and Miller, 1974 |
| Guppy, *Poecilia reticulata* | 8-12 mm | 24 | 69 | NR | NR | 0.0001 | 0.00001 | higher incidence of fin necrosis | Miller et al., 1979 |
| Northern Pike, *Esox lucius* | eggs and larvae | 96 | 23 | NR | NR | 0.0001 | ND | temporary inhibition of egg development | Helder, 1980 |
| Northern Pike, *Esox lucius* | eggs and larvae | 96 | 23 | 0.001 | 23 | 0.001 | 0.0001 | decreased survival and growth | Helder, 1980 |
| Frog, *Rana catesbiana* | larvae | i.p. injection | 50 | NR | NR | ND | 1000 μg/kg bw | no effect on survival metamorphosis, histology | Beatty et al., 1976 |
| Frog, *Rana catesbiana* | adults (150-250 g) | i.p. injection | 35 | NR | NR | 500 μg/kg bw | 250 μg/kg bw | temporary decrease in food consumption, but no effects on survival or histology | Beatty et al., 1976 |

[a] $LT_{50}$ = median lethal time in days after beginning exposure

[b] 47% mortality

NR = Not reported; ND = Not determined

Acute exposure experiments were also conducted by these researchers (Miller et al., 1973, 1979; Norris and Miller, 1974) with guppies, Poecilia reticulata. Miller et al. (1973) and Norris and Miller (1974) reported the effects of exposing guppies to nominal concentrations of 0.1, 1.0 and 10.0 μg/ℓ for 120 hours followed by transfer to clean water. Some fish (8-18%) died in each test concentration during the exposure period. All treated fish died by 37 days after beginning exposure; smaller fish generally died first. Fin necrosis was observed in all fish surviving more than 10 days. In a later study, Miller et al. (1979) measured the incidence of fin necrosis in guppies exposed for 24 hours to much lower nominal concentrations of 2,3,7,8-TCDD and then maintained for 69 days. The incidence of fin necrosis was significantly greater in fish exposed to ≥0.8 μg/kg bw (0.0001 μg/ℓ) than in controls or in fish exposed to 0.08 μg/kg bw (0.00001 μg/ℓ).

The effects of static acute exposure to 2,3,7,8-TCDD on eggs and larvae of northern pike, Esox lucius, and rainbow trout, Salmo gairdneri, were reported by Helder (1980) and Helder (1981), respectively. In both studies, newly fertilized eggs were exposed for 96 hours to a range of nominal 2,3,7,8-TCDD concentrations (0.0001, 0.0010, 0.010 μg/ℓ) followed by transfer to clean water. There was no significant increase in egg mortality up to the highest nominal test concentration of 0.010 μg/ℓ for either species. Significantly greater mortality occurred after hatching and during yolk sac absorption in both species at concentrations as low as 0.0010 μg/ℓ. Total mortality of pike fry reached 99% at 0.010 μg/ℓ and 50% at 0.0010 μg/ℓ by 23 days after fertilization. Total mortality of trout fry was 26% at 0.010 μg/ℓ and 12% at 0.0010 μg/ℓ. Although cumulative mortality was not significantly increased at the lowest test concentration (0.0001 µg/ℓ), sublethal effects occurred in both species. At this concentration, growth was significantly, but temporarily, retarded in both species.

Helder (1981) also exposed juvenile trout to nominal concentrations of 0.100 and 0.010 µg/ℓ for 96 hours and followed growth and survival for 72 days. Growth was significantly reduced in both groups. Mortality reached 100% by 27 days at the highest concentration, but was only 7% at the lowest concentration.

The only other study regarding the effects of acute exposure on aquatic animals is that of Beatty et al. (1976), who investigated the effects of single intraperitoneal injections of 2,3,7,8-TCDD in larval and adult frogs, Rana catesbiana. Groups of 15 tadpoles and 5 adults were injected with 2,3,7,8-TCDD in olive oil at maximum nominal dosages of 1000 and 500 µg/kg bw, respectively. There were no effects on survival and metamorphosis of larvae through 50 days after injection, or on survival of adults for 35 days after injection. There was a slight, temporary decrease in food consumption by adults at the highest dose. Histopathological examination revealed no significant lesions in metamorphosized or adult frogs. The lack of toxicity in this amphibian species is in sharp contrast to the results previously described with fish. Although the difference may be due, in part, to the different routes of exposure, it is probable that some fish are actually more sensitive, because toxic effects occurred in coho salmon at an internal dose of 5.4 µg/kg bw (Miller et al., 1973, 1979).

6.1.1.2. CHRONIC TOXICITY -- The effects of chronic or subchronic exposure to 2,3,7,8-TCDD have been reported for three species of freshwater invertebrates and three species of freshwater fish (Table 6-2). Miller et al. (1973) exposed adult snails, Physa sp., adult oligochoete worms, Paranais sp., and mosquito larvae Aedes aegypti to a nominal initial concentra-

TABLE 6-2

Effects of Chronic or Subchronic Exposure to 2,3,7,8-TCDD on Aquatic Animals

| Species | Life Stage, Weight or Length | Duration of Exposure (days) | Duration of Test (days) | Lowest Effect Concentration (µg/ℓ) | No Effect Concentration (µg/ℓ) | Effect | Reference |
|---|---|---|---|---|---|---|---|
| Mosquito, Aedes aegypti | larvae | 17 | 30 | ND | 0.2 | no effect on pupation | Miller et al., 1973 |
| Oligochaete Worm, Paranais sp. | adult | 55 | 55 | 0.2 | ND | reduced reproduction | Miller et al., 1973 |
| Snail, Physa sp. | adult | 36 | 48 | 0.2 | ND | reduced reproduction | Miller et al., 1973 |
| Snail, Helosoma sp. | adult | 32 | 46 | ND | 0.003 | no apparent effects | Yockim et al., 1978 |
| Waterflea, Daphnia magna | adult | 32 | 32 | ND | 0.003 | no apparent effects | Yockim et al., 1978 |
| Mosquitofish, Gambusia affinis | NR | 15 | 15 | 0.003 | ND | 100% mortality | Yockim et al., 1978 |
| Channel Catfish, Ictalurus punctatus | fingerlings | 20 | 20 | 0.003 | ND | 100% mortality | Yockim et al., 1978 |
| Rainbow Trout, Salmo gairdneri | 7.8 cm | 105 | 105 | 2300 µg/kg in diet | 2.30 µg/kg in diet | reduced survival, food consumption and growth, increased fin erosion | Hawkes and Norris, 1977 |

ND = Not determined

tion of 0.20 μg/ℓ for 36, 55 and 17 days, respectively. There was no significant difference in total pupation or pupation rate between exposed and control mosquito larvae during the 17-day exposure period or for the 30-day total test period. Exposure of adult snails to 0.20 μg/ℓ for 36 days had no significant effect on adult survival and egg production. The number of live juvenile snails and empty juvenile shells was counted 48 days after beginning exposure. The total snail hatch was ~30% lower (p=0.056) in the treated groups, but there was no significant difference in the percentage of survival of young snails. Exposure of worms to 2,3,7,8-TCDD resulted in a significant decrease in the total number of worms at 55 days. Total and mean dry weight were also reduced, but the variation among replicates reduced the statistical significance of this effect to p=0.057, indicating that 0.20 μg/ℓ exerted its principal effect on reproduction rather than individual worm growth.

Miller et al. (1973) also conducted chronic feeding studies with rainbow trout. The results of this study were also reported by Hawkes and Norris (1977). Groups of rainbow trout were fed diets containing 0.0023, 2.30 or 2300 μg/kg, 6 days/week for 105 days. The calculated doses were, respectively, 0.000032, 0.036 or 21.0 μg 2,3,7,8-TCDD/kg freeze-dry bw/day. Consumption of food containing 0.0023 and 2.3 μg/kg had no effect on survival, food consumption, growth and fin morphology. In contrast, fish fed the highest dose showed reduced food consumption after 10 days, reduced growth by 7 days, fin erosion by 14 days, and mortality that began on day 33 and reached 50% by day 61 and 88% by day 71.

The only other information concerning subchronic toxicity to aquatic animals was provided by Yockim et al. (1978), who exposed channel catfish, *Ictalurus punctatus*, mosquitofish, *Gambusia affinis*, waterfleas, *Daphnia* magna, snails, Helosoma sp., and algae, Oedogonium cardiacum, to $^{14}$C-labeled 2,3,7,8-TCDD in a recirculating aquatic model ecosystem. Soil was treated with 100 µg/kg and flooded with water, and organisms were added 1 day after flooding. Organisms were removed periodically for measurement of tissue residues. The mean concentration (µg/ℓ) in the water, measured by liquid scintillation counting, was 0.0034 at day 1, 0.0029 at day 3, 0.0024 at day 7, 0.0026 at day 15 and 0.0042 at day 32. The mean concentration through the 32-day period was 0.0031 µg/ℓ. No effects over the 32-day exposure period were observed in algae, waterfleas or snails as measured by reproductive activity, feeding and growth. All unharvested mosquitofish died by day 14, with a mean tissue concentration of 7.2 µg/kg bw. A second group of mosquitofish added at day 15 were all dead after 15-20 days. Channel catfish added at day 32 all died after 15-20 days of exposure, with a mean tissue concentration of 4.4 µg/kg bw. These results indicate that 15-20 days of exposure to ~0.003 µg/ℓ was lethal to fish, but had no effects on snails, waterfleas and algae.

6.1.1.3. AQUATIC PLANT EFFECTS -- As mentioned earlier, Yockim et al. (1978) did not observe any obvious effects of 0.003 µg/ℓ on the growth of the freshwater algae, O. cardiacum, over a 32-day period. The only other information concerning toxicity to aquatic plants was provided by Zullei and Benecke (1978), who conducted contact inhibition studies with filamentous algae, Phormidium sp. Filter paper was spotted in three places with 1 µg of 2,3,7,8-TCDD. Disks (5mm diameter) of filtered algae were placed on the spots, and the filter paper was placed in a petri dish containing nutrient media. The motility of the algae filaments outward from the disks was measured over a 3-hour period with a photoelectric cell. Relative to controls, 1 µg of 2,3,7,8-TCDD caused a significant inhibition of motility. Although the exposure concentration is unknown, these results indicate that this algal species may be affected by contact with contaminated substrates (i.e., sediment).

Jackson (1972) studied the progression of mitosis in the African blood lily, Haemanthus katherinae, endosperm cells. In this study, cells were exposed during prophase, prometaphase, metaphase and anaphase to 2,3,7,8-TCDD at nominal levels of either 0, 0.1 or 0.5 μg/ℓ, and the ability of the cells to progress to the next stage of cell division within a 2-hour period was evaluated. Regardless of the stage of cell division during which exposure occurred, the treatment resulted in an inhibition of progression to the next stage. The authors noted that 2,3,7,8-TCDD strongly adsorbs to glass and speculated that the concentrations in the test chamber were actually lower than reported. It was estimated that the higher concentration may possibly be approaching 0.2 μg/ℓ, the solubility of 2,3,7,8-TCDD in water.

## 6.2. TISSUE RESIDUES

Levels of 2,3,7,8-TCDD in several species of commercial fish taken from eastern Lake Ontario, Lake Erie and the Welland Canal ranged from 0.002-0.039 μg/kg in those fish with positive test results (Josephson, 1983). Rock bass showed no detectable levels. Highest concentrations generally occurred in eels (0.006-0.039 μg/kg), followed by smelt and catfish. The high fat content in these species (37, 13 and 3.5%, respectively) may explain, in part, the higher 2,3,7,8-TCDD concentrations.

Analysis by the NYS Department of Health showed levels of 2,3,7,8-TCDD in 46 muscle (fillet) samples of Lake Ontario fish that ranged from 0.002-0.162 μg/kg in 45 samples and were undetectable in one sample (NRCC, 1981a). The fish that were sampled included smallmouth bass, lake trout, white sucker, brown bullhead, rainbow trout, coho and chinook salmon, and brown trout. The Ontario Ministry of the Environment (NRCC, 1981a) reported concentrations of 2,3,7,8-TCDD ranging between 0.010 and 0.019 μg/kg in fillet samples of lake trout, brown trout, white bass, white perch and smelt in Lake Ontario, but no detectable (<0.010 μg/kg) levels in fish from the Niagara River, Lake Erie, Lake Huron or Lake Superior. Other fish residue data summarized by NRCC (1981a) included 2,3,7,8-TCDD concentrations in positive samples ranging from 0.020-0.230 μg/kg in Tittabawassee River, Saginaw Bay and other locations near Midland, MI; 0.015-0.480 μg/kg in the Arkansas River; and 0.019-0.102 μg/kg in Lake Ontario and Niagara River. OCDD concentrations in fish ranged from 0.040-0.150 μg/kg near Midland, MI, and from 0.004-0.078 μg/kg in the Honesatonic River. The levels of 2,3,7,8-TCDD in fish and shellfish as determined by various authors are given in Table 6-3.

Levels ranging from 0.004-0.695 μg/kg were cited by the U.S. EPA (1984) for the edible portion of channel catfish, carp, yellow perch, smallmouth bass, sucker and lake trout from Tittabawassee, Grand and Saginaw Rivers, Lake Michigan and Saginaw Bay. The highest concentrations were detected in bottom-feeding catfish and carp, and the lowest concentrations were detected in bass, perch and suckers (Harless and Lewis, 1980b).

Young et al. (1976) measured 2,3,7,8-TCDD residue levels in terrestrial and aquatic animals from contaminated areas of Eglin Air Force Base, FL, which had received massive amounts of herbicides, one of which (2,4,5-T) was contaminated with 2,3,7,8-TCDD. Beach mice from contaminated areas contained 0.540-1.30 μg/kg in the liver and 0.130-0.140 μg/kg in pelts. Residues in racerunner lizards trapped from the most highly contaminated

## TABLE 6-3

## Levels of 2,3,7,8-TCDDs in Fish and Shellfish

| Type/Section of Fish | Sampling Site | Concentration (ppt) | Reference |
|---|---|---|---|
| Edible flesh | Bayou Meto/Arkansas River | 480 | Mitchum et al., 1980 |
| Catfish | Bayou Meto/Arkansas River | ND (7 ppt)[a]-50 | Mitchum et al., 1980 |
| Buffalo | Bayou Meto/Arkansas River | ND (7-13 ppt)[a] | Mitchum et al., 1980 |
| Bottom feeder | Bayou Meto/Arkansas River | 77 | Mitchum et al., 1980 |
| Whole body | Tone River, Japan | 200 | Yamagishi et al., 1981 |
| Rock bass | Lake Ontario/Lake Erie/ Welland Canal | ND (<2 ppt)[a] | Josephson, 1983 |
| Eel, smelt and catfish | Lake Ontario/Lake Erie/ Welland Canal | 2-39 | Josephson, 1983 |
| Crayfish | Bergholtz Creek, Love Canal | 3.7 | Smith et al., 1983b |
| Catfish, bass and wall-eyed pike | 2,4,5-T contaminated watershed in Arkansas and Texas; Tittabawassee and Saginaw Rivers | ND (5-10 ppt)[a] | Shadoff et al., 1977;<br>U.S. EPA, 1980a;<br>Buser and Rappe, 1980 |
| Lake trout | Lake Ontario | 51-107 | O'Keefe et al., 1983 |
| Chinook salmon | Lake Ontario | 26-39 | O'Keefe et al., 1983 |
| Coho salmon | Lake Ontario | 20-26 | O'Keefe et al., 1983 |
| Rainbow trout | Lake Ontario | 17-32 | O'Keefe et al., 1983 |
| Brown trout | Lake Ontario | 8-162 | O'Keefe et al., 1983 |
| White perch | Lake Ontario | 17-26 | O'Keefe et al., 1983 |
| White sucker | Lake Ontario | ND (3.2)-10 | O'Keefe et al., 1983 |

TABLE 6-3 (cont.)

| Type/Section of Fish | Sampling Site | Concentration (ppt) | Reference |
|---|---|---|---|
| Smallmouth bass | Lake Ontario | 5.9 | O'Keefe et al., 1983 |
| Brown bullhead | Lake Ontario | 3.6 | O'Keefe et al., 1983 |
| Carp/Goldfish | Cayuga Creek | 87 | O'Keefe et al., 1983 |
| Northern pike | Cayuga Creek | 32 | O'Keefe et al., 1983 |
| Pumpkin seed | Cayuga Creek | 31 | O'Keefe et al., 1983 |
| Rock bass | Cayuga Creek | 12 | O'Keefe et al., 1983 |
| Coho salmon | Lake Erie | 1.4-<3.5 | O'Keefe et al., 1983 |
| Walleye pike | Lake Erie | 2.6 | O'Keefe et al., 1983 |
| Smallmouth bass | Lake Erie | 1.6-<2.4 | O'Keefe et al., 1983 |
| Carp/Goldfish | Lake Erie | ND (2.6) | O'Keefe et al., 1983 |
| Lake trout | Lake Huron | 21 | O'Keefe et al., 1983 |
| Carp | Lake Huron | 26 | O'Keefe et al., 1983 |
| Channel catfish | Lake Huron | 20 | O'Keefe et al., 1983 |
| Sucker | Lake Huron | 25 | O'Keefe et al., 1983 |
| Yellow perch | Lake Huron | ND (8.7) | O'Keefe et al., 1983 |
| Coho salmon | Lake Michigan | ND (3.8) | O'Keefe et al., 1983 |
| Rainbow trout | Lake Superior | 1.0 | O'Keefe et al., 1983 |
| Perch/sucker | Saginaw Bay | ND (3.8)-25 | Niemann et al., 1983 |
| Catfish | Saginaw Bay | 14-37 | Niemann et al., 1983 |
| Carp | Saginaw Bay | 23-47 | Niemann et al., 1983 |

TABLE 6-3 (cont.)

| Type/Section of Fish | Sampling Site | Concentration (ppt) | Reference |
|---|---|---|---|
| Catfish | Bayon Meto/Arkansas River | ND (3.8) | Niemann et al., 1983 |
| Bottom feeders | Bayon Meto/Arkansas River | ND (6.7)-12 | Niemann et al., 1983 |
| Lake trout | Lake Ontario | 34-54 | Niemann et al., 1983 |
| Rainbow trout | Lake Ontario | 43 | Niemann et al., 1983 |
| Ocean haddock | Atlantic Ocean | ND (4.6) | Niemann et al., 1983 |
| Carp | Lake Huron | 3-28 | Stalling et al., 1983 |
| Carp | Saginaw Bay | 94 | Stalling et al., 1983 |
| Carp | Bay Port | 27 | Stalling et al., 1983 |
| Carp | Tittabawassee River | 81 | Stalling et al., 1983 |
| Lake trout | Lake Michigan | 5 | Stalling et al., 1983 |
| Brown trout | Lake Ontario | 33 | Stalling et al., 1983 |
| Yellow perch | Woods Pond, MA | 26 | Buser and Rappe, 1983 |
| Channel catfish | Tittabawassee River, Saginaw River and Grand River | 157 (13)[c] | Harless and Lewis, 1982 |
| Carp | Tittabawassee River, Saginaw River and Grand River | 55 (7)[c] | Harless and Lewis, 1982 |
| Yellow perch | Tittabawassee River and Saginaw River | 13 (5)[c] | Harless and Lewis, 1982 |
| Small mouth bass | Grand River | 8 (6)[c] | Harless and Lewis, 1982 |

TABLE 6-3 (cont.)

| Type/Section of Fish | Sampling Site | Concentration (ppt) | Reference |
|---|---|---|---|
| Sucker | Tittabawassee River and Saginaw Bay | 10 (4)[c] | Harless and Lewis, 1982 |
| Trout | Lake Michigan | ND (5)[c] | Harless and Lewis, 1982 |
| Trout | Lake Ontario at Burlington, Canada | 61.2 (3.6) | Ryan et al., 1983 |
| Trout | Lake Ontario at Toronto Harbor, Canada | 32.3 (3.6) | Ryan et al., 1983 |
| Trout | Lake Huron at Burnt Island, Canada | 30.4 (3.6) | Ryan et al., 1983 |

[a] Not detected and the detection limit is indicated within the parentheses.

[b] Only the GC/MS results of these authors are included in tabulation

[c] These are the mean concentrations in samples showing detectable levels of 2,3,7,8-TCDD.

ND = Not detected

areas contained 0.36-0.37 μg/kg in the visceral mass and trunk, respectively. Residues were also found in three fish species taken from a stream and pond in the contaminated area. Residue levels of 0.012 μg/kg were found in the viscera of sailfin shiners and in the bodies (heads and tails removed) of mosquitofish. Samples of skin, muscle, gonad and gut of spotted sunfish contained 0.004, 0.004, 0.018 and 0.085 μg/kg 2,3,7,8-TCDD, respectively. 2,3,7,8-TCDD was not detected in insect larvae, snails, diving beetles, crayfish, tadpoles and other fish species taken from waterbodies that contained 0.010-0.035 μg/kg in the sediments.

Finally, the levels of 2,3,7,8-TCDD in wildlife have been determined by various authors. These values are shown in Table 6-4. From the somewhat higher levels of 2,3,7,8-TCDD found in Saginaw Bay and in Lake Ontario gull eggs (Table 6-4), Norstrom et al. (1982) indicated the possibility of industrial contamination since the former is near a major 2,4,5-T manufacturing plant on the Saginaw/Tittabawassee River, and the latter is downstream from a 2,4,5-TCP plant at Niagara Falls, NY.

## 6.3. ECOSYSTEM EFFECTS

Investigations concerning the ecosystem effects of 2,3,7,8-TCDD are restricted to the field studies of Young et al. (1975) at the Eglin Air Force Base. A 1-square mile area was sprayed with massive amounts of herbicides over an 8-year period (1962-1970). In particular, a 92-acre test area was sprayed from 1962-1964 with 87,186 pounds of 2,4,5-T that was contaminated with 2,3,7,8-TCDD. Analysis in 1974 of surface soils in this area showed 2,3,7,8-TCDD levels of 0.010-0.710 μg/kg. Large numbers of beach mice were trapped from contaminated and control sites and evaluated for differences in organ weights and histopathology. The only significant differences in organ weight were increased liver weight in females and

## TABLE 6-4

## TCDD Levels in Wildlife

| Type of Animal | Tissue | Sampling Site | 2,3,7,8-TCDD Concentration (ppb) | | Reference |
|---|---|---|---|---|---|
| | | | Average$^a$ | Range | |
| Rabbit | liver | Seveso, Italy | 31 | 1-<1024 | Fanelli et al., 1980a |
| Field mouse | whole body | Seveso, Italy | 4.5 | 0.07-49 | Fanelli et al., 1980c |
| Hare | liver | Seveso, Italy | 7.7 | 2.7-13 | Fanelli et al., 1980c |
| Toad | whole body | Seveso, Italy | 0.2 | LS | Fanelli et al., 1980c |
| Snake | liver | Seveso, Italy | 2.7 | LS | Fanelli et al., 1980c |
| Snake | adipose tissue | Seveso, Italy | 16 | LS | Fanelli et al., 1980c |
| Earthworm | whole body | Seveso, Italy | 12 | LS | Fanelli et al., 1980c |
| Eagle | carcass | throughout U.S. | <50 ppb | NR | Helling et al., 1973 |
| Herring gull | egg | Saginaw Bay, Lake Ontario | NR | 0.043-0.093 | Ogilvie, 1981 |

TABLE 6-4 (cont.)

| Type of Animal | Tissue | Sampling Site | 2,3,7,8-TCDD Concentration (ppb) | | Reference |
|---|---|---|---|---|---|
| | | | Average[a] | Range | |
| Herring gull | egg | Lake Superior | 0.011 | NR | Norstrom et al., 1982 |
| Herring gull | egg | Lake Michigan | 0.009 | NR | Norstrom et al., 1982 |
| Herring gull | egg | Lake Huron (main body) | 0.009 | NR | Norstrom et al., 1982 |
| Herring gull | egg | Lake Huron, Saginaw Bay, N. | 0.043 | NR | Norstrom et al., 1982 |
| Herring gull | egg | Lake Huron, Saginaw Bay, S. | 0.086 | NR | Norstrom et al., 1982 |
| Herring gull | egg | Lake Erie | 0.011 | NR | Norstrom et al., 1982 |
| Herring gull | egg | Lake Ontario | 0.059 | NR | Norstrom et al., 1982 |
| Turtle | egg and liver | Bayou Meto/ Arkansas River | 0.15 | LS | Mitchum et al., 1980 |
| Snake | liver and muscle | Bayou Meto/ Arkansas River | 0.060 | LS | Mitchum et al., 1980 |
| Muskrat | liver | Bayou Meto/ Arkansas River | ND (40 ppt)[b] | LS | Mitchum et al., 1980 |

TABLE 6-4 (cont.)

| Type of Animal | Tissue | Sampling Site | 2,3,7,8-TCDD Concentration (ppb) |  | Reference |
|---|---|---|---|---|---|
|  |  |  | Average[a] | Range |  |
| Racoon | liver | Bayou Meto/ Arkansas River | ND (10 ppt)[b] | LS | Mitchum et al., 1980 |
| Frog | liver and muscle | Bayou Meto/ Arkansas River | >10 | LS | Mitchum et al., 1980 |
| Horse | fat | Midwest wire reclamation incinerator | 0.045 | LS | Hryhorczuk et al., 1981 |
| Horse | liver | Midwest wire reclamation incinerator | ND (<6 ppt)[b] | LS | Hryhorczuk et al., 1981 |

[a] These are averages of samples that had above detectable levels of TCDD.

[b] Not reported and the limit of detection indicated in parentheses

NR = Not reported; LS = Limited samples

increased spleen weight in males and females taken from the contaminated sites; however, no histopathological effects could be attributed to the collection sites. Similar studies on racerunner lizards showed no significant difference in relative or total body weight of animals collected from contaminated and control sites. Sweep net surveys of the contaminated sites for terrestrial insects in 1971 and 1973 indicated that there was a significant increase in the number of families and total number of insects in the contaminated test site, which was correlated with the increase in vegetation after herbicide spraying. Aquatic species diversity studies were conducted in 1969, 1970, 1973 and 1974 on a stream in the contaminated area and a control stream. As mentioned before, 2,3,7,8-TCDD was detected in sediments and fish from the contaminated stream; however, there was no significant difference in ichthyofauna diversity in the two streams, and no significant change in diversity through time in either stream. As a result, the only effects that can be attributed to 2,3,7,8-TCDD contamination were increased liver and spleen weight in beach mice. The ecological significance of this effect is unknown, especially since no obvious detrimental effects were observed in this or other species from contaminated sites.

Korfmacher et al. (1984) analyzed fat tissue and eggs from snakes for 2,3,7,8-TCDD. Water snakes were selected as a possible marker for 2,3,7,8-TCDD contamination. Three snakes were collected from Lake Dupree, Arkansas in 1983. This lake is a site of 2,3,7,8-TCDD contaminated sediment and fish (Arkansas Dept. of Pollution Control and Ecology, 1983). Two snakes were collected from a lake evidently not contaminated with 2,3,7,8-TCDD from any industrial source. Eggs were derived from one of the snakes obtained from Lake Dupree. 2,3,7,8-TCDD concentration in the fat material of three snakes from contaminated lake varied from 500-730 ppt, in the snake eggs varied from 151-294 ppt, and in the fat material from two snakes from noncontaminated lake varied from 38-378 ppt.

The only other information pertinent to ecosystem level effects was provided by Bollen and Norris (1979), who investigated the effects of 2,3,7,8-TCDD on respiration ($CO_2$ production) in forest litter and soil samples. Litter and soil samples were air dried, placed in biometer flasks, moistened and treated with 2,3,7,8-TCDD. Concentrations as high as 0.031 μg/kg dry weight in litter had no effect on respiration. Concentrations as high as 0.052 μg/kg dry weight in soil caused a slight but significant stimulation of $CO_2$ production. Because higher concentrations were not tested, it is unknown whether 2,3,7,8-TCDD would have inhibitory effects on soil microbial populations, carbon metabolism or nutrient cycling at the higher levels of soil contamination found in such contaminated areas as the Eglin Air Force Base test site.

## 6.4. SUMMARY

Almost all of the available information concerning the toxicity of PCDDs to wildlife deals with aquatic species. Acute exposure to initial nominal 2,3,7,8-TCDD concentrations as low as 0.0001 μg/ℓ has been shown to cause delayed sublethal effects in early life stages of northern pike and rainbow trout (Helder 1980, 1981) and in adult guppies (Miller et al., 1979). Decreased growth, food consumption and survival have been reported in these and other fish species after acute exposure to $\geq$0.001 μg/ℓ. During these tests, the nominal initial concentrations probably decreased rapidly because of uptake by test organisms, adsorption to the exposure containers and perhaps volatilization. As a result, it is possible that constant acute or chronic exposure to dissolved concentrations <0.0001 μg/ℓ would produce toxic effects in sensitive aquatic organisms.

Several studies provide evidence that 2,3,7,8-TCDD is less toxic to aquatic invertebrates and amphibians than to the tested fish species. Subchronic exposure to an initial nominal concentration of 0.20 μg/ℓ had no effect on mosquito population and caused a 30-50% decrease in reproduction of snails and oligochoete worms (Miller et al., 1973). In contrast, acute exposure to 0.1 μg/ℓ caused 100% delayed mortality in guppies (Norris and Miller, 1974) and juvenile rainbow trout (Helder 1981). Similarly, exposure to relatively constant, measured, dissolved concentrations of ~0.002-0.004μg/ℓ in aquatic model ecosytems killed all exposed mosquitofish and channel catfish in 15-20 days, but had no discernible effects on snails and waterfleas over a total test period of 32-46 days (Yockim et al., 1978). The dying mosquitofish and catfish had mean whole-body 2,3,7,8-TCDD concentrations of 7.2 and 4.4 μg/kg, respectively. In contrast, single intraperitoneal injections of 2,3,7,8-TCDD at maximum doses of 500 or 1000 μg/kg bw, respectively, had no effects on adult frogs over a 35-day period or on frog larvae over a 50-day period (Beatty et al., 1976).

Chronic feeding studies with groups of rainbow trout showed that daily feeding of 2300 μg/kg in the diet was lethal to all but two fish (88%) in 71 days, but no significant effects were seen in fish fed daily a diet containing 2.3 μg/kg for 105 days (Hawkes and Norris, 1977). Residue analysis of single fish sampled at the end of the tests showed 2,3,7,8-TCDD levels of 1380 μg/kg bw in one high dose fish and 1.573 μg/kg in one low dose fish.

Although only limited information was found concerning the effects of 2,3,7,8-TCDD on aquatic plants, it is probable that they are less sensitive than fish. Using model ecosystems, Yockim et al. (1978) observed no obvious effects on algae at concentrations (0.002-0.004 g) that killed fish. Zullei and Benecke (1978) observed contact inhibition of filamentous algae placed in contact with 1 µg quantities of 2,3,7,8-TCDD spotted on filter paper.

The only available information concerning the effects of low level environmental exposure to 2,3,7,8-TCDD on terrestrial wildlife was reported by Young et al. (1975), who investigated tissue residues and several biological parameters in mice and lizards from contaminated and control sites at Eglin Air Force Base, FL. The concentrations of 2,3,7,8-TCDD in contaminated soils were 0.010-0.710 µg/kg. Mice trapped from the contaminated site contained 0.540-1.30 µg/kg in the liver and had significantly higher spleen and liver weights than mice from control sites. No other differences (histopathology, weights of other organs, incidence of abnormal fetuses, etc.) were observed. Racerunner lizards from the contaminated site contained 0.36-0.37 µg/kg in the viscera and trunk and showed no differences in body weight or histopathology compared with lizards from control sites. Residues of 2,3,7,8-TCDD in three fish species taken from a pond and stream adjacent to the contaminated site ranged from 0.004-0.085 µg/kg. Sediments derived from the erosion taken from the contaminated site contained localized concentrations of 0.010-0.035 µg/kg. PCDD residues have been reported for numerous other fish species and other snakes from contaminated water bodies. The PCDD concentrations (primarily 2,3,7,8-TCDD) in positive fish tests ranged from 0.002-0.695 µg/kg.

# 7. COMPOUND DISPOSITION AND RELEVANT PHARMACOKINETICS

## 7.1. ABSORPTION

Data are available regarding the absorption of 2,3,7,8-TCDD through the gastrointestinal (GI) tract and skin of experimental animals. Absorption through the respiratory tract, however, has not been studied. Also, there are no data on the absorption of 2,3,7,8-TCDD when mixed with other chlorinated compounds, which is presumably the case for human exposures.

7.1.1. Absorption from the Gastrointestinal Tract. Data on the GI absorption of 2,3,7,8-TCDD are summarized in Table 7-1. The GI absorption of 2,3,7,8-TCDD has been investigated more extensively in the rat than in other species. When 2,3,7,8-TCDD was administered in the diet at 7 or 20 ppb for 42 days, 50-60% of the consumed dose was absorbed (Fries and Marrow, 1975). Administration of 2,3,7,8-TCDD by gavage in acetone:corn oil (1:25 or 1:9) as a single dose or as repeated doses (5 days/week x 7 weeks) resulted in absorption of a larger percentage (70-86%) of the dose (Rose et al., 1976; Piper et al., 1973). It would appear, therefore, that the GI absorption of 2,3,7,8-TCDD may vary, depending upon the vehicle used. The influence of vehicle or adsorbent on GI absorption has been investigated by Poiger and Schlatter (1980), using hepatic concentrations 24 hours after dosing as an indicator of the amount absorbed. They found a linear relationship between ng 2,3,7,8-TCDD administered by gavage in 50% ethanol (for doses of 12-280 ng, equivalent to 0.06-1.4 μg/kg) and the percentage of the dose in hepatic tissues (36.7-51.5%). At the next higher dose of 1070 ng the percentage was 42%. Administration of 2,3,7,8-TCDD in an aqueous suspension of soil resulted in a decrease in the hepatic levels of 2,3,7,8-TCDD as compared with hepatic levels resulting from administration of

## TABLE 7-1

## Gastrointestinal Absorption of 2,3,7,8-TCDD

| Species | Vehicle | Dose Schedule (μg/kg) | % Absorption Mean ± SD | Reference |
|---|---|---|---|---|
| Guinea pig | NR | NR single dose | 50 | Nolan et al., 1979 |
| Rat | 7 ppb, in diet | 0.5 μg/kg/day x 42 days | 50 - 60 | Fries and Marrow, 1975 |
| Rat | 20 ppb, in diet | 1.4 μg/kg/day x 42 days | 50 - 60 | Fries and Marrow, 1975 |
| Rat | A:C, 1:25 | 1.0 μg/kg, single dose | 84 ± 11* | Rose et al., 1976 |
| Rat | A:C, 1:25 | 0.1 or 1.0 μg/kg/day, 5 days/week x 7 weeks | 86 ± 12* | Rose et al., 1976 |
| Rat | A:C, 1:9 | 50.0 μg/kg, single dose | 70 | Piper et al., 1973 |
| Hamster | olive oil | 650 μg/kg, single dose | 74 ± 23* | Olson et al., 1980a |

*Mean ± standard deviation

NR = Not reported; A:C = Acetone:corn oil, v:v

2,3,7,8-TCDD in 50% ethanol. The extent of the decrease was directly proportional to the length of time the 2,3,7,8-TCDD had been in contact with the soil. McConnell et al. (1984) observed a dose-response relation of liver accumulation of 2,3,7,8-TCDD as a result of intragastric exposure of young male Hartley guinea pigs to 2,3,7,8-TCDD in corn oil or in soil (Table 7-2). In Sprague-Dawley female rats, they found as high as 40.8 ppb and 20.3 ppb liver accumulation of 2,3,7,8-TCDD by intragastic exposure to 2,3,7,8-TCDD in corn oil and in soil, respectively. Philippi et al. (1981) and Huetter and Philippi (1982) have shown that radiolabeled 2,3,7,8-TCDD becomes progressively more resistant with time to extraction from soil. Poiger and Schlatter (1980) also demonstrated that 2,3,7,8-TCDD mixed in an aqueous suspension of activated carbon was very poorly absorbed (<0.07% of the dose in hepatic tissues). In addition, Silkworth et al. (1982) observed an increase in the $LD_{50}$ value for female guinea pigs from 2.5 to 19 µg/kg when the 2,3,7,8-TCDD was administered by gavage in corn oil or aqueous methyl cellulose, respectively.

A comparative study on the biological uptake in the rabbit of 2,3,7,8-TCDD in different formulations, including accident-contaminated Seveso soil, was conducted by Bonaccorsi et al. (1983). On the whole, the results indicated that soil-borne 2,3,7,8-TCDD had a bioavailability lower than that of free (solvent-borne) 2,3,7,8-TCDD.

The feeding of fly ash containing PCDDs to rats in the diet for 19 days resulted in considerably lower hepatic levels of PCDDs than did the feeding of an extract of the fly ash at comparable PCDD dietary concentrations (Van der Berg et al., 1983). The PCDDs were tentatively identified as 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD. The

TABLE 7-2

Liver Accumulation of 2,3,7,8-TCDD in Guinea Pigs
30 Days after a Single Intragastric Exposure to 2,3,7,8-TCDD[a]

| Group | No. of Animals | Composition of the Material Gavaged | Total Quantity Gavaged | Dosage of TCDD (μg/kg bw) | Average Liver Concentration of TCDD[b] ppt ± SEM |
|---|---|---|---|---|---|
| 1 | 6 | Corn oil | 0.1 mℓ/100 g | 0 | ND |
| 2 | 6 | TCDD in corn oil | 0.1 mℓ/100 g | 1 | 1.6±0.2<br>4.1[c] |
| 3 | 6 | TCDD in corn oil | 0.1 mℓ/100 g | 3 | 13.3±2.3 |
| 4 | 6 | Time Beach soil | 0.35 g | 1.3 | <1.0 |
| 5 | 5[d] | Time Beach soil | 1.07 g | 3.8 | 1.0±0.1<br>3.2[c] |
| 6 | 5[e] | Time Beach soil | 3.60 g | 12.8 | 34.3±6.0 |
| 7 | 6 | Minker Stout soil | 0.26 g | 1.1 | <1.0 |
| 8 | 6 | Minker Stout soil | 0.80 g | 3.3 | 1.4±0.3<br>2.0±0.1[c] |
| 9 | 6 | Minker Stout soil | 2.67 g | 11.0 | 25.7±5.2 |
| 10 | 5[e] | Time Beach soil (uncontaminated) | 3.60 g | 0 | ND |

TABLE 7-2 (cont.)

| Group | No. of Animals | Composition of the Material Gavaged | Total Quantity Gavaged | Dosage of TCDD (μg/kg bw) | Average Liver Concentration of TCDD[b] ppt ± SEM |
|---|---|---|---|---|---|
| 11 | 6 | Time Beach soil (uncontaminated but TCDD added) | 2.71 g | 10 | 45.4+8.4 |

[a] Source: McConnell et al. (1984)

[b] Detection limit 100 ppt

[c] Animal/animals which died before 30 days

[d] One animal died 2 days after dosing (not included)

[e] One animal died at the time of dosing

SEM = Standard error of the mean

ND = Not detected

difference in hepatic levels noted between fly ash-treated and extract-treated rats was greater for the more highly chlorinated isomers than it was for 2,3,7,8-TCDD.

The GI absorption of 2,3,7,8-TCDD was also examined in the hamster, the species most resistant to the acute toxicity of this toxin. Olson et al. (1980a) administered a single, sublethal, oral dose of [1,6-$^{3}$H]-2,3,7,8-TCDD in olive oil (650 μg/kg) to hamsters and reported that 74% of the dose was absorbed, while Nolan et al. (1979) reported that absorption in the guinea pig, the most sensitive species, was ~50% following administration of an unspecified amount of 2,3,7,8-TCDD. The vehicle and method for calculating the absorbed dose were not given in this report.

7.1.2. Absorption Through the Skin. Information on the absorption of 2,3,7,8-TCDD through the skin is extremely limited. Poiger and Schlatter (1980) administered 26 ng 2,3,7,8-TCDD in 50 μℓ methanol to the skin of six rats. After 24 hours, the liver contained 14.8±2.6% of the dose. By comparing with hepatic levels obtained (in the same study) after oral administration in 50% ethanol (see Section 7.1.1.), assuming that hepatic levels are valid estimates of the amount absorbed from both oral and dermal routes and that absorption from methanol is equivalent to absorption from 50% ethanol, the amount absorbed from a dermal application can be estimated at ~40% of the amount absorbed from an equivalent oral dose. As compared with dermal application in methanol, dermal application of 2,3,7,8-TCDD to rats in vaseline or polyethylene glycol resulted in hepatic tissue concentration of 1.4 and 9.3% of the dose, respectively, but had no observable effect on the concentration of 2,3,7,8-TCDD required to induce skin lesions (~1 μg) in the rabbit ear assay (Poiger and Schlatter, 1980). Application of 2,3,7,8-TCDD in a soil/water paste decreased hepatic 2,3,7,8-TCDD to ~2% of the administered dose and increased the amount required to produce skin lesions to 2-3 μg in rats and rabbits, respectively. Application in an activated carbon/water paste essentially completely eliminated absorption, as measured by percent of dose in the liver, and increased the amount of 2,3,7,8-TCDD required to produce skin lesions to ~160 μg.

## 7.2. DISTRIBUTION

The tissue distribution of 2,3,7,8-TCDD in a number of species is summarized in Table 7-3. As would be predicted from the lipophilic nature of this compound, accumulation tends to occur in tissues with a high lipid content. In rats and mice, 2,3,7,8-TCDD residues are localized in the liver and adipose tissue. In the rat, hepatic levels of 2,3,7,8-TCDD accounted for ~38-52% of the administered dose during the first week following oral administration of a single dose ranging from 0.07-50 μg/kg (Piper et al., 1973; Poiger and Schlatter, 1979). The latter dose is within the $LD_{50}$ range for rats. Similar results were obtained 7 days following administration of a single intraperitoneal dose of 400 μg/kg of [$^3$H]2,3,7,8-TCDD to rats; 43% of the total dose was localized in the liver (Van Miller et al., 1976). In two strains of mice, the liver contained ~35% of an administered dose of 2,3,7,8-TCDD 1 day after oral or intraperitoneal administration (Manara et al., 1982). In both species, 1-22 days after single-dose oral or intraperitoneal administration, levels of 2,3,7,8-TCDD in adipose tissue were similar to or slightly lower than levels in the liver, and were considerably higher than concentrations in other tissues (Piper et al., 1973; Rose et al., 1976; Van Miller et al., 1976; Manara et al., 1982), including the thymus (Rose et al., 1976; Van Miller et al., 1976).

In a 7-week gavage study and a 2-year dietary study of 2,3,7,8-TCDD in rats, 2,3,7,8-TCDD was present in the liver at 3-5 times the concentration in adipose tissue when the daily dose or intake of the compound was $\geq$0.01 μg/kg/day (Rose et al., 1976; Kociba et al., 1976) and was present at

## TABLE 7-3

## Distribution of 2,3,7,8-TCDD

| Species | Route of Administration | Principal Organ Depots | Reference |
|---|---|---|---|
| Rat | oral | liver | Fries and Marrow, 1975 |
| Rat | oral | liver > fat | Rose et al., 1976 |
| Rat | oral | liver > fat | Piper et al., 1973 |
| Rat | oral | liver > fat | Kociba et al., 1978a |
| Rat | oral | liver > fat | Allen et al., 1975 |
| Rat | i.p. | liver > fat | Van Miller et al., 1976 |
| Mouse | oral | liver > fat > kidney > lung | Manara et al., 1982 |
| Mouse | i.p. | liver > fat > kidney > lung > spleen | Manara et al., 1982 |
| Rhesus monkey | i.p. | fat > skin > liver > adrenals = thymus | Van Miller et al., 1976 |
| Golden Syrian hamster | i.p. or oral | liver > fat | Olson et al., 1980a |
| Guinea pig | oral | fat > liver > adrenals > thymus > skin | Nolan et al., 1979 |
| Guinea pig | i.p. | fat > liver > skin | Gasiewicz and Neal, 1979 |

i.p. = intraperitoneal

about the same concentration as in adipose tissue when the daily intake was 0.001 μg/kg/day (Kociba et al., 1976). As in the single-dose studies, 2,3,7,8-TCDD levels were considerably lower in other tissues, including the thymus, than in liver or adipose tissue (Rose et al., 1976).

There is some evidence of sex differences in tissue distribution in rats. During 42 days of administration of 2,3,7,8-TCDD at 7 or 20 ppb in the diet, ~85% of the total body residue of male rats was located in the liver, as compared with 70% in females (Fries and Marrow, 1975). This small difference in distribution patterns may have resulted from sex differences in relative adipose tissue content.

The ability of mouse liver to sequester 2,3,7,8-TCDD increases with prolonged exposure (Teitelbaum and Poland, 1978). The hepatic uptake of $[^3H]$2,3,7,8-TCDD in Swiss-Webster mice was maximal 12 hours after intraperitoneal injection. Hepatic uptake, expressed as percent of total dose, increased from 11.7% in control mice to 60.9% in mice that had been pretreated with a single dose of unlabeled 2,3,7,8-TCDD 36 hours previously. This observation is consistent with other data that indicate that 2,3,7,8-TCDD is a potent inducer of hepatic microsomal mixed-function oxidase (Section 8.1.1.5.) and that >90% of the hepatic 2,3,7,8-TCDD is localized in the microsomes (Allen et al., 1975). The toxicity of 2,3,7,8-TCDD in mice has been demonstrated to correlate with the affinity of the receptor that controls this induction in mice (Poland and Glover, 1980).

In nonhuman primates, the liver seems to have much less of a role in 2,3,7,8-TCDD accumulation. Van Miller et al. (1976) have compared the tissue distribution of $[^3H]$2,3,7,8-TCDD in adult rhesus monkeys, infant rhesus monkeys, and Sprague-Dawley rats 7 days after a single intraperitoneal injection of 400 μg 2,3,7,8-TCDD/kg bw. They found that while 43% of the administered dose was localized in the livers of the rats, only 10.4%

was found in the livers of adult monkeys and 4.5% in the livers of infant monkeys. This difference cannot be explained by differences in absorption or excretion, since these parameters were observed to be similar in both species. In monkeys, larger percentages of the dose were found in adipose tissue, skin and muscle than was the case for rats.

McNulty et al. (1982) reported that 2 years after administration of a single oral dose of 1 μg/kg of 2,3,7,8-TCDD to an adult rhesus macaque monkey, tissue levels of the compound were 1000 ppt in adipose tissue and 15 ppt in the liver. These results indicate that prolonged retention of 2,3,7,8-TCDD may occur in this species. The tissue distribution of 2,3,7,8-TCDD in the guinea pig appears to be similar to the monkey, with the highest concentration of the toxin being found in adipose tissue (Gasiewicz and Neal, 1979; Nolan et al., 1979). The interspecies difference in the tissue distribution of 2,3,7,8-TCDD may be related to the relative adipose tissue content of a given species and the affinity of 2,3,7,8-TCDD for the hepatic microsomal fraction; however, the significance of these differences remains in doubt. For example, the hepatotoxicity of 2,3,7,8-TCDD in a given species does not appear to be related to the hepatic concentration of the toxin (Neal et al., 1982).

Very limited data are available on the tissue distribution of 2,3,7,8-TCDD in humans. Facchetti et al. (1980) reported tissue concentrations of 2,3,7,8-TCDD at levels of 1-2 ng/g in adipose tissue and pancreas, 0.1-0.2 ng/g in liver and <0.1 ng/g in thyroid, brain, lung, kidney and blood in a woman who died 7 months after potential exposure to 2,3,7,8-TCDD from the Seveso accident. This pattern of 2,3,7,8-TCDD distribution, however, may not be representative for humans since the woman at the time of death had an adenocarcinoma (which was not considered related to the accident) that involved the pancreas, liver and lungs.

In addition, Young et al. (1983) reported preliminary results of the analyses of adipose tissue from soldiers exposed to Agent Orange. Two analyses were performed, one using the exact mass of 321.8936 and the other the signal profile at masses of 321.8936 and 319.8965. Three groups were studied consisting of 20 veterans claiming health problems related to Agent Orange exposure; 3 Air Force officers with known heavy exposure to Agent Orange during disposal operations and 10 control veterans with no known herbicide exposure. In the first group, 10 of the 20 had measurable levels of 2,3,7,8-TCDD (5 with 5-7 ppt, 3 with 9-13 ppt, 1 with 23 and 35 ppt and another with 63 and 99 ppt). In the second group, only two officers had measurable 2,3,7,8-TCDD levels that did not exceed 3 ppt. In the 10 control veterans, 4 had 2,3,7,8-TCDD levels between 6 and 14 ppt. Levels of 2,3,7,8-TCDD in adipose tissue did not appear to be associated in this study with ill health or any particular symptom; however, it was considered that information on background levels of 2,3,7,8-TCDD in adipose tissue was too limited to draw any firm conclusions.

2,3,7,8-TCDD has been demonstrated to be fetotoxic in the rat (Section 9.1.). The ability of 2,3,7,8-TCDD to gain access to the developing fetus of Fischer 344 rats following a single oral dose of [$^{14}$C]2,3,7,8-TCDD was investigated by Moore et al. (1976). They found low concentrations of 2,3,7,8-TCDD in the fetus at gestation days 14, 18 or 21. The radioactivity appeared to be evenly distributed throughout the fetus on days 14 and 18; however, increased levels of radioactivity were detected in fetal liver on day 21.

Nau and Bass (1981) (more recently reported by Nau et al., 1982) investigated the fetal uptake of 2,3,7,8-TCDD in NMRI mice following oral, intraperitoneal or subcutaneous administration of 5, 12.5 or 25 μg/kg in

DMSO:corn oil or acetone:corn oil. The chemical was usually administered as a single dose 2 days before sacrifice. Embryonic 2,3,7,8-TCDD concentrations were maximal on gestational days 9 and 10; however, low levels were found in the embryo and fetus between gestational days 11 and 18. This sharp decrease in 2,3,7,8-TCDD concentration coincides with placentation. 2,3,7,8-TCDD concentrations in the placenta were an order of magnitude greater than in the fetus itself. The affinity of fetal liver for 2,3,7,8-TCDD was relatively low, as compared with maternal liver; however, 2,3,7,8-TCDD levels in fetal livers were 2-4 times higher than the levels in other fetal organs. An attempt was made to correlate 2,3,7,8-TCDD levels in the fetuses with the observed incidence of cleft palate, but no clear relationship was observed (i.e., 5 minutes to 61 days after injection).

Autoradiographic studies of tissue localization following intravenous administration of [$^{14}$C]2,3,7,8-TCDD in DMSO to three strains of mice indicated that the liver had the highest concentration and longest retention of radioactivity in the body, followed by the nasal mucosa (Appelgren et al., 1983). In pregnant mice, the concentration of radioactivity in the fetuses was lower than in the dams, but a similar, selective labelling of the liver and the nasal mucosa was seen in the fetuses at day 17 of gestation. In the adult animals, labelling of the adrenal cortex was about equal to that of the liver at 1 hour after dosing, but thereafter was much lower than in the liver. Labelling of the thymus, lymph nodes, bone marrow and prostate were low at all observation times.

## 7.3. METABOLISM

Vinopal and Casida (1973) found no evidence of water soluble metabolites of 2,3,7,8-TCDD following incubation with mammalian liver microsomes or intraperitoneal injection into mice. In the same experiment, only unmetabolized 2,3,7,8-TCDD was extractable from mouse liver 11-20 days after treatment. Piper et al. (1973), however, detected $^{14}$C activity in the expired air and urine within the first 10 days following administration to rats, indicating that some metabolic alteration of 2,3,7,8-TCDD occurs. Nelson et al. (1977) found that incubation of [$^{14}$C]2,3,7,8-TCDD with rat hepatic microsomes resulted in the formation of bound radioactivity which, in contrast to free 2,3,7,8-TCDD, was not ethyl acetate extractable. This binding was found to result from oxidative metabolism, as indicated by a requirement for NADPH, and could be induced by phenobarbital pretreatment. Binding was not covalent, because the bound radioactivity could be extracted with chloroform:methanol (9:1); this extracted radioactivity cochromatographed with the 2,3,7,8-TCDD standard.

Ramsey et al. (1982) detected five distinct radioactive compounds in the bile of rats given daily oral doses of 15 µg [$^{14}$C]2,3,7,8-TCDD. Incubation of the bile with φ-glucuronidase resulted in an increase in the amount of [$^{14}$C] extracted, implying the existence of conjugated [$^{14}$C]-2,3,7,8-TCDD metabolites. All of the 2,3,7,8-TCDD-derived radioactivity in the bile corresponded to metabolized 2,3,7,8-TCDD. *In vivo* metabolism has also been detected in the Golden Syrian hamster (Olson et al., 1980a) and in dogs (Poiger et al., 1982a). In urine and bile from $^{14}$C-TCDD treated rats, hamsters and guinea pigs, all of the radioactivity corresponded to metabolites of TCDD, as assessed by HPLC (Neal et al., 1982). Enzymatic hydrolysis of the TCDD metabolites present in urine and bile produced alterations in their HPLC profiles that indicated the presence of glucuronide conjugates in bile and sulfate conjugates in urine (Olson and Bittner, 1983).

The ability of 1,6-$^3$H-2,3,7,8-TCDD derived radioactivity to bind to rat hepatic macromolecules *in vivo* was investigated by Poland and Glover (1979). They found maximum levels of 60 pmol 2,3,7,8-TCDD/mole of amino acids in protein, 12 pmol 2,3,7,8-TCDD/mole of nucleotide in rRNA, and 6 pmol of 2,3,7,8-TCDD/mole of nucleotide in DNA. According to the authors this corresponds to one 2,3,7,8-TCDD-DNA adduct/35 cells (Poland and Glover, 1979). Similar results were obtained using a mouse liver microsomal system (Guenthner et al., 1979a). [$^3$H]2,3,7,8-TCDD was found to bind to microsomal protein 120-2640 times more readily than to deproteinized salmon sperm DNA. They estimated the rate of 2,3,7,8-TCDD metabolism to be between 9000 and 36,000 times lower than the rate of P-450-mediated benzo[a]pyrene metabolism.

Tulp and Hutzinger (1978) studied the metabolism of a variety of PCDDs, including 1,2,3,4-TCDD, in the rat. In di- and higher substituted dioxins, only mono- and dihydroxy derivatives were detected. Primary hydroxylation occurred exclusively at the 2-, 3-, 7- or 8-position, so the significance of this study for the metabolism of 2,3,7,8-TCDD is not clear. Sawahata et al. (1982) investigated the metabolism of 2,3,7,8-TCDD in isolated rat hepatocytes. The major product was deconjugated with φ-glucuronidase, derivatized with diazomethane, and separated into two compounds by HPLC. These metabolites were subsequently identified as 1-hydroxy-2,3,7,8-TCDD and 2-hydroxy-3,7,8-trichlorodibenzo-*p*-dioxin.

Poiger et al. (1982a) identified six metabolites in the bile of dogs that were given [$^3$H]2,3,7,8-TCDD. The major metabolite was 1,3,7,8-tetrachloro-2-hydroxydibenzo-*p*-dioxin. 2-Hydroxy-3,7,8-trichlorodibenzo-*p*-dioxin and 1,2-dichloro-4,5-dihydroxybenzene were also identified as minor metabolites. The structures of the three remaining metabolites were not determined; however, two appeared to be trichloro-dihydroxydibenzo-p-dioxins and the third was apparently a chlorinated 2-hydroxydiphenyl ether. The presence of these metabolites is consistent with a 1,2-arene oxide intermediate.

Isolated rat hepatocytes in suspension have been used as an in vitro system for assessing 2,3,7,8-TCDD metabolism under various conditions. Data indicate that the rate of 2,3,7,8-TCDD metabolism in rat hepatocytes correlates directly with drug induced changes in hepatic cytochrome P-450 monooxygenase activity, suggesting that 2,3,7,8-TCDD is metabolized by this enzyme (Olson et al., 1981).

Beatty et al. (1978) found a correlation between hepatic mixed-function oxidase (MFO) activity and the toxicity of 2,3,7,8-TCDD in rats. Both in naturally occurring age- and sex-related differences in MFO activity and following the administration of inducers and inhibitors of MFO enzyme systems, hepatic MFO activity was inversely related to toxicity that corresponds to direct relationship between the 20-day $LD_{50}$ and MFO activity.

The fate of 2,3,7,8-TCDD metabolites from dogs has been examined in rats by Weber et al. (1982). 2,3,7,8-TCDD metabolites were extracted from the bile of 2,3,7,8-TCDD-treated dogs and administered by gavage to female Sprague-Dawley rats. The 2,3,7,8-TCDD metabolites were rapidly cleared from the bodies of bile-duct-cannulated rats, with >85% of the dose recovered in the feces, bile and urine within 24 hours. In intact rats, only 13% of the dose was excreted in the feces and urine during the first 24 hours, indicating enterohepatic circulation; however, the administered radioactivity was completely eliminated within 72 hours after dosing.

Poiger et al. (1982a) investigated the toxicity of 2,3,7,8-TCDD metabolites by administering bile extract from 2,3,7,8-TCDD-treated dogs to male guinea pigs in single oral doses equivalent to 0.6, 6.0 and 60 μg of parent compound/kg bw. Other groups of guinea pigs received bile extract from untreated dogs or 2,3,7,8-TCDD itself. A comparison of the mortality data at 5 weeks after dosing indicated that the acute toxicity of 2,3,7,8-TCDD to guinea pigs was at least 100 times higher than was the acute toxicity of its metabolites.

Olson and Bittner (1983) reported that the rate of metabolite formation _in vitro_ was considerably higher in hepatocytes from the hamster than in hepatocytes from the rat. Qualitative evaluation of _in vivo_ and _in vitro_ metabolites by HPLC also suggested major interspecies variability. The authors suggested that such differences in metabolism may partially explain the differences in toxicity among species.

## 7.4. ELIMINATION

The following discussion assumes that elimination is a first order process. With the exception of the guinea pig, which may follow zero order kinetics (Gasiewicz and Neal, 1979), elimination data yield a straight line on a semilogarithmic plot, indicating a first order process. Hiles and Bruce (1976) pointed out that the studies of Allen et al. (1975) and Piper et al. (1973) can be interpreted equally well by either zero or first order kinetics. The majority of the data, however, seem to support the assumption of a first order elimination process.

2,3,7,8-TCDD is slowly excreted from the bodies of all species tested (Table 7-4), with a half-life in the body of 10-43 days. In the Golden Syrian hamster, the least sensitive mammalian species to the acute toxicity of 2,3,7,8-TCDD, excretion occurs readily through both the urine (41%) and

TABLE 7-4

Elimination of 2,3,7,8-TCDD

| Species | Single Treatment µg/kg (route) | Half-Life for Elimination (days) | Relative % of TCDD-Derived Radioactivity | | Reference |
|---|---|---|---|---|---|
| | | | Feces | Urine | |
| Guinea pig | 2 (i.p.) | 30.2 ± 5.8 | 94.0 | 6.0 | Gasiewicz and Neal, 1979 |
| Guinea pig | 1.45 (oral) | 22 - 43 | NT | NT | Nolan et al., 1979 |
| Rat | 1.0 (oral) | 31 ± 6 | >99 | <1 | Rose et al., 1976 |
| Rat | 50 (oral) | 17.4 ± 5.6 | 80.0 | 20.0 | Piper et al., 1973 |
| Rat | 50 (oral) | 21.3 ± 2.9 | 95.5 | 4.5 | Allen et al., 1975 |
| Rat | 400 (i.p.) | NT | 91.0 | 9.0 | Van Miller et al., 1976 |
| Monkey (adult) | 400 (i.p.) | NT | 78.0 | 22.0 | Van Miller et al., 1976 |
| Monkey (infant) | 400 (i.p.) | NT | 39.0 | 61.0 | Van Miller et al., 1976 |
| Monkey | 1 (oral) | 365 | NR | NR | McNulty et al., 1982 |
| Mouse | | | | | |
| C57BL/65 | 10 (i.p.) | 11.0 ± 1.2 | 72.0 | 28.0 | Gasiewicz et al., 1983a,b |
| DBA/2J | 10 (i.p.) | 24.4 ± 1.0 | 54.0 | 46.0 | Gasiewicz et al., 1983a,b |
| B6D2$F_1$/J* | 10 (i.p.) | 12.6 ± 0.8 | 72.0 | 28.0 | Gasiewicz et al., 1983a,b |
| Hamster | 650 (i.p.) | 10.8 ± 2.4 | 59.0 | 41.0 | Olson et al., 1980a |
| Hamster | 650 (oral) | 15.0 ± 2.5 | NT | NT | Olson et al., 1960a |

*Offspring of C57BL/6J and DBA/2J that are heterozygous at the Ah locus

NT = Not tested; NR = not reported

feces (59%) (Olson et al., 1980a). The high levels found in the urine of infant monkeys were probably due to the incomplete separation of urine and feces (Van Miller et al., 1976). In all the other species so far tested, excretion occurs mainly through the feces (80-100%) with only minor amounts of 2,3,7,8-TCDD metabolites found in the urine (Piper et al., 1973; Allen et al., 1975; Rose et al., 1976; Gasiewicz and Neal, 1979).

Rose et al. (1976) investigated the elimination of [$^{14}$C]2,3,7,8-TCDD in rats given repeated oral doses of 0.01, 0.1 or 1.0 μg/kg/day Monday through Friday for 7 weeks, or a single dose of 1.0 μg/kg. In these studies, no $^{14}$C was excreted in the urine following a single dose; however, the urine contained 3-18% of the cumulative dose by 7 weeks. This study indicated that steady-state concentrations will be reached in the bodies of rats in ~13 weeks. The rate constant defining the approach to steady-state concentrations was independent of the dosage of 2,3,7,8-TCDD over the range studied. This is consistent with the observations of Fries and Marrow (1975), who found that the total retention in the bodies of rats was proportional to total intake. When rats were maintained on a diet containing either 7 or 20 ppb TCDD, the amount of TCDD retained in the body was 5.5 times the daily intake of TCDD at 14 days, 7.5 times the daily intake at 28 days, and 10.0 times the daily intake at 42 days.

The data in Table 7-4 suggest some interspecies differences in the half-life for elimination ($t_{1/2}$) of 2,3,7,8-TCDD. In the hamster, the least sensitive species to the acute toxicity of 2,3,7,8-TCDD, a mean $t_{1/2}$ of 10.8 days was observed (Olson et al., 1980a,b), and in the guinea pig, the most sensitive species to the acute toxicity of 2,3,7,8-TCDD, the mean $t_{1/2}$ was 30.2 days (Gasiewicz and Neal, 1979). The observed interspecies differences in the $t_{1/2}$ of 2,3,7,8-TCDD may in part be related to the relative sensitivity of a given species to the acute toxicity of 2,3,7,8-TCDD.

The intrastrain differences in the $t_{1/2}$ of 2,3,7,8-TCDD in three mouse strains may be due to the finding that the DBA/2J strain possesses ~2-fold greater adipose tissue stores than the C57Bl/6J and $B6D2F_1$/J strains (Gasiewicz et al., 1983b). The sequestering of the lipophilic toxin in adipose tissue stores of the DBA/2J mouse may contribute to the greater persistence of 2,3,7,8-TCDD in this strain.

In all of the rat studies shown in Table 7-4, urinary and fecal elimination were monitored for a period of only 20-22 days, and from these data it was assumed that elimination followed a single component, first order kinetic model. Recently, Olson and Bittner (1983) examined the elimination of 2,3,7,8-TCDD-derived radioactivity in rats over a 35-day period following a single intraperitoneal exposure at 1 μg $^3$H-2,3,7,8-TCDD/kg. They observed first order kinetics for elimination, with a fast component having a $t_{1/2}$ of 7 days (represents 13% of total elimination) and a slow component having a $t_{1/2}$ of 75 days (87% of total). The second, slow component for elimination was evident only when urinary and fecal elimination were monitored for >30 days. This study suggests that 2,3,7,8-TCDD may be more persistent than earlier studies suggested. A preliminary study in the rhesus monkey suggests that 2,3,7,8-TCDD may be exceptionally persistent in adipose tissue. McNulty et al. (1982) estimated the apparent half-life of 2,3,7,8-TCDD in the fat of a monkey to be ~1 year.

Studies in the rat, guinea pig, hamster and mouse have found that all of the 2,3,7,8-TCDD-derived radioactivity excreted in the urine and bile corresponds to metabolites of 2,3,7,8-TCDD (Neal et al., 1982, 1984). The apparent absence of 2,3,7,8-TCDD metabolites in liver and fat suggests that once formed, the metabolites of 2,3,7,8-TCDD are readily excreted. Thus, urinary and biliary elimination of 2,3,7,8-TCDD is apparently dependent upon metabolism of the toxin. Although urine and bile appear to be free of unmetabolized 2,3,7,8-TCDD, data from the hamster and rat indicate that a significant amount (10-40%) of unchanged 2,3,7,8-TCDD may be excreted into the feces. Unmetabolized 2,3,7,8-TCDD thus appears to enter the intestinal lumen by some route other than bile for a number of days following treatment. These data suggest that the in vivo half-life for elimination of 2,3,7,8-TCDD may not directly reflect the rate of 2,3,7,8-TCDD metabolism in a given animal (Neal et al., 1982, 1984). These data are consistent with the observation of Manara et al. (1982) that the lethal effects of 2,3,7,8-TCDD were decreased in C57Bl/6J mice regardless of whether the compound was administered by gavage or intraperitoneal injection if the animals were given diets containing activated carbon.

## 7.5. SUMMARY

Exposure to 2,3,7,8-TCDD occurs by inhalation, dermal or GI absorption. Inhalation exposure to detectable levels of 2,3,7,8-TCDD is less likely because of low vapor pressure of this compound; however, inhalation exposure could result from inhalation of mist, dust or other contaminated particulate matter. Monitoring of atmospheric dust in the Seveso area detected 2,3,7,8-TCDD levels ranging from 0.06-2.1 ng 2,3,7,8-TCDD/g airborne dust (DiDomenico et al., 1980b). This corresponds to an estimated 24-hour inhalation exposure of 1.4 pg assuming an average intake of 10 $m^3$ air containing 0.14 mg dust/$m^3$. No studies on the systemic absorption of 2,3,7,8-TCDD have been performed, so the significance of this route of exposure in contaminated areas cannot be assessed.

2,3,7,8-TCDD is readily absorbed under experimental conditions (*vide ante*) and following environmental contamination (Cockerham et al., 1980; Fanelli et al., 1980c; Walsh, 1977). After being absorbed, 2,3,7,8-TCDD is rapidly distributed to tissues with a high lipid content (fat, skin, adrenals). In most species studied, the major storage site for 2,3,7,8-TCDD is the liver (see Table 7-3). 2,3,7,8-TCDD exposure results in induction of MFO activity and a proliferation of smooth endoplasmic reticulum, the major subcellular storage site for 2,3,7,8-TCDD (Section 8.1.1.5.). The ability of 2,3,7,8-TCDD to produce this effect has been correlated with the sensitivity of various strains of mice to 2,3,7,8-TCDD toxicity (Van Miller et al., 1976; Poland and Glover, 1980).

2,3,7,8-TCDD appears to be distributed throughout the body and stored largely as the parent compound (Olson et al., 1980a); however, metabolism to more polar compounds appears to be necessary for excretion in the urine or bile (Weber et al., 1982; Olson et al., 1980a; Neal et al., 1984). Studies have also indicated that 2,3,7,8-TCDD was metabolized by the hepatic cytochrome P-450 monooxygenase system. The structures of six metabolites in the dog (Poiger et al., 1982b) and two in the rat (Sawahata et al., 1982) have been elucidated; however, the structure of the metabolites of 2,3,7,8-TCDD have not been determined for the other species studied. Although some [1,6-$^3$H]-2,3,7,8-TCDD-derived radioactivity was capable of binding covalently to cellular macromolecules (Guenthner et al., 1979b; Nelson et al., 1977; Poland and Glover, 1979), metabolism of 2,3,7,8-TCDD seems to be predominantly a detoxification process (Beatty et al., 1978; Poiger et al., 1982a).

2,3,7,8-TCDD and its metabolites are excreted from the body by a variety of mechanisms. Lactating rats excrete 2,3,7,8-TCDD in the milk (Moore et al., 1976). 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD and 1,2,3,4,6,7,8-HpCDD and OCDD have been detected in human milk samples from Swedish and German mothers (Rappe et al., 1985). These investigators could detect 1,2,3,4,7,8-, 1,2,3,6,7,8- and 1,2,3,7,8,9-HxCDDs only in the mothers' milk from Sweden. Piper et al. (1973) reported the excretion of $[^{14}C]$2,3,7,8-TCDD-derived radioactivity in the feces, urine and expired air of rats given a single oral dose of 50 μg/kg. Over a 21-day period, 53, 13 and 3% of the administered radioactivity was eliminated through the feces, urine and expired air, respectively. This pattern of excretion seems typical of most species studied, with the exception of the hamster, which was observed to excrete 41% of the 2,3,7,8-TCDD-derived radioactivity in the urine (Olson et al., 1980a). In all species so far studied, metabolism and excretion are relatively slow processes, with the observed initial half-lives in experimental animals on the order of a few weeks (see Table 7-4).

# 8. TOXICOLOGY: ACUTE, SUBCHRONIC AND CHRONIC

## 8.1. EXPERIMENTAL ANIMALS

### 8.1.1. Acute.

8.1.1.1. LETHAL EFFECTS -- There have been studies in a variety of species defining the doses necessary to cause death after acute exposure to 2,3,7,8-TCDD. A summary of the single dose $LD_{50}$ data for 2,3,7,8-TCDD is presented in Table 8-1. The dose that results in death varies extensively with species, with the male guinea pig being the most sensitive species tested ($LD_{50}$ of 0.6 μg/kg) (Schwetz et al., 1973), and the male hamster the least sensitive species tested ($LD_{50}$ of 5051 μg/kg) (Henck et al., 1981). The rat and monkey appear to be the second most sensitive species, with $LD_{50}$s between 22 and 70 μg/kg (Schwetz et al., 1973; McConnell et al., 1978a), while other species tested (rabbit and mouse) had $LD_{50}$s between 114 and 283 μg/kg (Schwetz et al., 1973; McConnell et al., 1978b; Vos et al., 1974). Schwetz et al. (1973) found male rats more sensitive to 2,3,7,8-TCDD, while Beatty et al. (1978) found adult female and weanling male rats more sensitive than adult male rats (Table 8-1). In C57Bl/10 mice, Smith et al. (1981) reported adult males to be far more sensitive to the acute toxicity of 2,3,7,8-TCDD than adult females. Thus, data on sex differences in sensitivity to the acute toxicity of 2,3,7,8-TCDD are conflicting and may depend on the species or strain examined.

Harris et al. (1973) studied the toxic effects of 2,3,7,8-TCDD in rats, mice and guinea pigs with regard to single or multiple exposures. Similar effects were observed after a single exposure to 2,3,7,8-TCDD as were observed when multiple exposures totaled the same dose as received in the single exposure. As illustrated most clearly in rats, a single dose of 25 μg/kg, 6 weekly doses of 5 μg/kg, or 30 daily doses of 1 μg/kg were

## TABLE 8-1

## Lethal Doses of 2,3,7,8-TCDD Following Acute Exposure

| Species/Strain | Sex/No./Group | Route/ Vehicle | Dose Tested (μg/kg) | Duration of Observation | $LD_{50}$ (μg/kg) | Comments | Reference |
|---|---|---|---|---|---|---|---|
| Guinea pigs/ Hartley | M/NR | gavage/corn oil-acetone (9:1) | NR | 2-8 weeks | 0.6 (0.4-0.9)* | Time to death was 5-34 days, the 2,3,7,8-TCDD was 91% pure | Schwetz et al., 1973 |
| Guinea pigs/ Hartley | M/NR | gavage/corn oil-acetone (9:1) | NR | 2-8 weeks | 2.1 (1.5-3)* | Time to death was 9-42 days, the 2,3,7,8-TCDD was 99% pure | Schwetz et al., 1973 |
| Guinea pigs/ Hartley | M/9 | gavage/ corn oil | NR | 30 days | 2 | Median time to death was 17-20 days, marked weight loss, thymus atrophy, intestinal hemorrhage, no porphyria and only mild liver injury | McConnell et al., 1978b |
| Guinea pigs/ Hartley | F/6 | gavage/ corn oil | 0.1<br>0.5<br>2.5<br>12.5<br>20.0 | 42 days | 2.5 (1.2-5.4, 95% confidence) | Time to first death was 32 days in the 2.5 μg/kg group, with 50% mortality by day 42 | Silkworth et al., 1982 |
| Guinea pigs/ Hartley | F/6 | gavage/ methyl cellulose | 0.1<br>0.5<br>2.5<br>12.5<br>20.0 | 12 days | 19 (15-23, 95% confidence) | Time to first death was 12 days in the 20.0 μg/kg group, with 67% mortality by day 42 | Silkworth et al., 1982 |
| Rats/ Sherman | M/5-10 | gavage/corn oil-acetone (9:1) | 8<br>16<br>32<br>63 | 2-8 weeks | 22 | Time to death was 9-27 days, the 2,3,7,8-TCDD was 91% pure | Schwetz et al., 1973 |
| Rats/ Sherman | F/NR | gavage/corn oil-acetone (9:1) | NR | 2-8 weeks | 45 (30-66)* | Time to death was 13-43 days, the 2,3,7,8-TCDD was 91% pure | Schwetz et al., 1973 |
| Rats/Sprague-Dawley | M/6 | i.p./olive oil | NR | 20 days | 60 | $LD_{50}$ (μg/kg, mean ± SE) adult male, 60.2 ± 7.8; weanling male, 25.2 ± 1.4 | Beatty et al., 1978 |
| Rats/Sprague-Dawley | F/6 | i.p./olive oil | NR | 20 days | 25 | Adult female had a mean ± SE of 24.6 ± 2.0 μg/kg | Beatty et al., 1978 |

## TABLE 8-1 (cont.)

| Species/Strain | Sex/No./Group | Route/ Vehicle | Dose Tested (μg/kg) | Duration of Observation | LD$_{50}$ (μg/kg) | Comments | Reference |
|---|---|---|---|---|---|---|---|
| Monkey/rhesus | F/3 | gavage/ corn oil | 0<br>70<br>350 | >35 days | <70 | Weight loss, edema, severe thymus atrophy, loss of hair, mild liver damage | McConnell et al., 1978a |
| Mice/C57Bl | M/14 | gavage/corn oil-acetone (9:1) | 0<br>100<br>150<br>200 | 60 days | 114 | Time to death in the high dose group was 15-20 days, bw loss, edema in 25% of treated animals, severe thymic and spleen atrophy, hemorrhage in the region of the eye and small intestine, liver necrosis in the centrilobular region | Vos et al., 1974 |
| Mice/C57Bl | M/9 | gavage/ corn oil | NR | 30 days | 283.7 | Median time to death was 22-25 days, dose-related bw loss, thymic atrophy, increased liver weight and porphyria, gross and historic liver alterations, subcutaneous edema, intestinal hemorrhage | McConnell et al., 1978b |
| Mice/C57Bl/10 | M/5 | gauge/ arachis oil | 85<br>107<br>135<br>170<br>213 | 45 days | 146 | 95% confidence limits of 111-211 μg/kg. Most deaths occurred from 22-26 days after dosing. Signs of porphyria, edema, hemorrhage. | Smith et al., 1981 |
| Mice/C57Bl/10 | F/5 | gauge/ arachis oil | 85<br>107<br>135<br>170<br>213<br>269<br>338<br>426<br>536 | 45 days | >450 | 1 of 4 animals died at dose of 425 μg/kg | Smith et al., 1981 |
| Mice/C57Bl/6J | M/NR | i.p./olive oil | NR | 30 days | 132 | BGD2F$_1$/J mice are the offspring of C57Bl/6J and DBA/2J. | Gasiewicz et al., 1983a,b |
| Mice/DBA/2J | M/NR | i.p./olive oil | NR | 30 days | 620 | The BGD2$_1$/J mice are heterozygous at the Ah locus. | Gasiewicz et al., 1983a,b |
| Mice/B6D2F$_1$/J | M/NR | i.p./olive oil | NR | 30 days | 300 | No comment | Gasiewicz et al., 1983a,b |

TABLE 8-1 (cont.)

| Species/Strain | Sex/No./Group | Route/ Vehicle | Dose Tested ($\mu$g/kg) | Duration of Observation | $LD_{50}$ ($\mu$g/kg) | Comments | Reference |
|---|---|---|---|---|---|---|---|
| Rabbits/ New Zealand | M&F/NR | gavage/corn oil-acetone (9:1) | NR | 2-8 weeks | 115 (38-345)* | Time to death was 6-39 days, the 2,3,7,8-TCDD was 91% pure | Schwetz et al., 1973 |
| Rabbits/ New Zealand | M&F/5 | i.p./ corn oil | 32<br>63<br>126<br>252<br>500 | 4 weeks | NR | Time to death was 6-23 days, 2-3 animals/group died in all but the low exposure group | Schwetz et al., 1973 |
| Rabbits/ New Zealand | M&F/NR | dermal/ acetone | 31.6<br>63<br>126<br>252<br>500 | 3 weeks | 275 (142-531)* | Time to death was 12-22 days | Schwetz et al., 197[illegible] |
| Hamster/ golden Syrian | M/6 | gavage/corn oil-acetone (9:1) | 0<br>300<br>600<br>1000<br>3000<br>6000 | 55 days | 5051 (3876-18,487, 95% confidence) | Time to death was 26-43 days, the liver and thymus appeared to be the primary target organs, only 1 death occurred in the 300 and 3000 $\mu$g/kg group | Henck et al., 1981 |
| Hamster/ golden Syrian | M&F/5-6 | i.p./ olive oil | 0<br>500<br>1000<br>2000<br>3000 | 50 days | >3000 | Significant, dose-related decrease in thymus weight starting at 500 $\mu$g/kg, only 2 deaths occurred out of 11 hamsters in the 3000 $\mu$g/kg group. | Olson et al., 1980b |
| Hamster/ golden Syrian | M/5 | gavage/ olive oil | 500<br>1000<br>2000<br>3000 | 50 days | 1157 | Death generally occurred between 24 and 45 days, decrease in bw above 2000 $\mu$g/kg, proliferative ileitis with mild to severe inflammation | Olson et al., 1980b |
| Dogs/Beagle | M/2 | gavage/corn oil-acetone (9:1) | 3000 | 2-8 weeks | NA | All animals died | Schwetz et al., 1973 |
| Dogs/Beagle | F/2 | gavage/corn oil-acetone (9:1) | 30<br>100 | 2-8 weeks | NA | All animals survived | Schwetz et al., 1973 |

*The number in parentheses appears to indicate the range of lethal doses; however, the article did not specify what these numbers represented.

i.p. = Intraperitoneal; NR = Not reported; NA = Not applicable all the threshold dose for observing a decrease in body weight. In general, other endpoints, including lethality, decrease in thymus weight, and a no effect level for body weight change in rats, mice and guinea pigs required a specific threshold level regardless of whether this level was achieved through a single exposure or a small number of multiple exposures.

Although 2,3,7,8-TCDD has over a $10^3$-fold difference in toxicity depending upon the species tested, some of the signs of lethal toxicity were the same regardless of species. One of the most characteristic observations after acute lethal exposure to 2,3,7,8-TCDD was the protracted time between exposure and death (see Table 8-1). In determining the $LD_{50}$ in the least sensitive animal, the hamster, the test animals died between 24 and 45 days after a single acute exposure (Olson et al., 1980b), and similar observations were made in all other species tested including the most sensitive species, the guinea pig, in which animals died up to 42 days after treatment (Schwetz et al., 1973).

During this extended period between treatment and death the animals had poor weight gain or loss of weight resulting in a "wasting syndrome" that resembled starvation. Though weight loss is the primary general feature observed in adult rats, in the young animals depletion of body fat results in lean tissue formation (Peterson et al., 1984) In female Wistar rats intubated with 2,3,7,8-TCDD at a dose of 100 µg/kg, the weight loss was biphasic (Courtney et al., 1978). The initial weight loss occurred rapidly during the first 7-10 days after treatment and was associated with decreased food and water consumption. This initial phase of weight loss was reversed with the resumption of normal food intake for 4 or 5 days, only to be followed by a second, more gradual, decline in food and water intake and weight until death. Providing animals with an adequately nutritious liquid diet by intubation did not appreciably alter the pattern of weight loss nor affect survival. In contrast, Gasiewicz et al. (1980) observed that providing rats with total parenteral nutrition would prevent some of the weight loss induced by 2,3,7,8-TCDD; however, there was no protection from the lethal effects of 2,3,7,8-TCDD. Seefeld and Peterson (1983) and Seefeld et al. (1984) found that a reduction in food intake caused by 2,3,7,8-TCDD is primarily responsible for the loss of body weight or depressed growth rate of rats. Pair-fed control rats lost weight at the same rate and to the same extent as their weight-matched 2,3,7,8-TCDDtreated partners (25 or 50 µg/kg) until day 10 after treatment. At 20-35 days after treatment, the body weight of the two groups began to diverge, with the pair-fed control group having body weights that were 20-30 g higher than the corresponding 2,3,7,8-TCDD groups. The mortality in the 25 and 50 µg/kg groups was 33 and 75%, respectively, while in the corresponding pair-fed groups the mortality was 0 and 15%. The authors proposed a hypothesis that 2,3,7,8-TCDD lowers a regulated level or "set-point" for body weight control in the rat. The ensuing change in food intake was thought to occur secondarily to the change in set-point (Seefeld and Peterson, 1983; Seefeld et al., 1984; Peterson et al., 1984). Vitamin A or E did not protect or inhibit the decrease in body weight, respectively. Further, these vitamins provided little protection against 2,3,7,8-TCDD-induced lethality in rats (Hassan et al., 1985).

Also, severe thymic atrophy is universally observed in all species given lethal doses of 2,3,7,8-TCDD, and since weight loss and thymic atrophy are both associated with malnutrition, van Logten et al. (1981) investigated the effects of dietary protein on the toxicity of 2,3,7,8-TCDD. Groups of female Fischer 344 rats administered 2,3,7,8-TCDD (20 µg/kg) and maintained on low (3.5%), normal (26%) or high (55%) protein diets maintained approximately the same amount of weight (-0.2±3, 7±6 and 7±3 g for each dietary group, respectively) during the subsequent 10-day period. The weight gain in treated animals was 10-18 g less than that in the respective control rats. Dietary protein also had no effect on preventing or enhancing the 2,3,7,8-TCDD induced thymic atrophy. Although weight loss and thymic atrophy were present in most species tested, there were other symptoms that were characteristic of toxicity in only some species.

In the guinea pig, besides thymic atrophy, no gross changes were observed in internal organs after a lethal oral or i.p. dose of 2,3,7,8-TCDD (Greig et al., 1973, Gupta et al., 1973). Hemorrhages were observed in a number of organs including the adrenal gland, urinary bladder, GI tract and mesenteric lymph nodes; however, these were considered unremarkable changes by Gupta et al. (1973). Histologic examination confirmed the gross observations with atrophy and lymphoid cell depletion in the thymus, spleen and lymph nodes, and hemorrhages observed in many organs. In addition, marked hyperplasia of the urinary bladder was observed. Of particular interest was the absence of severe toxic effects on the liver. Gross observation under UV light indicated no excess of porphyrin, while histologic examinations revealed diffuse single cell necrosis. Identical observations were made by McConnell et al. (1978b) in guinea pigs administered lethal doses of 2,3,7,8-TCDD, with the additional observation that the sternal bone marrow was hypocellular in all types of blood-forming cells.

Turner and Collins (1983) described some histologic changes in the liver of guinea pigs treated with 2,3,7,8-TCDD. Groups consisting of 4-6 female Hartley guinea pigs were treated with 2,3,7,8-TCDD at doses of 0.0, 0.1, 0.5, 2.5, 12.5 or 20 μg/kg, and 1 male guinea pig each was treated with a dose of 0.1 or 0.5 μg/kg. The 2,3,7,8-TCDD was administered by gavage as an aqueous suspension in 0.75% methyl cellulose and surviving animals were killed 42 days after treatment. A second group of guinea pigs (6 males and 6 females/dose) were administered soot generated from a fire in a transformer cooled by polychlorinated biphenyls and chlorinated benzenes (1, 10, 100 and 500 mg/kg). The histologic observations as described were applied in general to both treatment groups and there was no apparent relationship between dose and response. At the light microscope level, hepatocellular hypertrophy, steatosis, focal necrosis, cytoplasmic degeneration and acidophilic hyalin-like cytoplasmic inclusion bodies were observed. Even though there was no dose-response relationship for these liver lesions, the doses spanned a range that resulted in the lowest dose being nonlethal (none of the 4 female guinea pigs died during the study), while in the high dose group 4 of 6 animals died before 42 days post-treatment. The $LD_{50}$ for female guinea pigs was determined in this study to be 2.5 or 19 μg/kg bw depending on whether the compound was administered by gavage in corn oil or in aqueous methyl cellulose (Silkworth et al., 1982).

The greatest difference at necropsy in the gross and histologic effects in rats and mice of exposure to lethal doses of 2,3,7,8-TCDD was pathologic alterations in the liver, as compared with guinea pigs. An early report by Buu-Hoi et al. (1972) described alterations in the architecture of the liver of rats within 5 days of receiving a low dose of 2,3,7,8-TCDD (10 μg/kg by i.p. injection). At higher oral doses of 100 or 50 μg/kg, which killed 43 and 7% of the animals, respectively, Gupta et al. (1973) also observed marked distortion of liver architecture in rats; however, only mild regenerative changes of the liver were observed at the sublethal dose of 5 μg/kg administered weekly for 6 weeks. Liver toxicity appeared to develop slowly in the rat with no change in liver function, as indicated by plasma protein and bilirubin levels, or alkaline phosphatase, glutamic-oxalacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) activity being detected 3 days after intubation with 2,3,7,8-TCDD at a dose of 200 μg/kg (Greig et al., 1973). Bilirubin levels were, however, markedly elevated from 0.33 μg/100 mℓ in control animals to 10.97 μg/100 mℓ in treated animals 21 days after exposure (the other parameters were not measured at this time, although plasma protein was slightly but significantly decreased when determined 9 days post-treatment). As in rats, the livers of mice exposed to lethal levels of 2,3,7,8-TCDD had signs of necrotic changes (Vos et al., 1974); however, Jones and Grieg (1975) reported that the centrilobular necrosis, bile duct proliferation and lipid accumulation were more extreme in mice than in rats. Examination of mouse livers using long wave UV light showed fluorescence suggestive of excess porphyrin accumulation (McConnell et al., 1978b). Although excess porphyrins may be present in the livers from 2,3,7,8-TCDD-exposed rats, fluorescence is not usually observed.

Besides effects on the liver, 2,3,7,8-TCDD exposure produced other toxic effects in rats and mice that were not observed or were observed to a lesser extent in guinea pigs. In rats that died from 2,3,7,8-TCDD exposure, there were extensive hemorrhages of the heart, liver, brain, adrenal gland and GI tract along with ulcers and necrosis of the glandular stomach, and in females, atrophy of the uterus (Gupta et al., 1973). In mice, facial edema was severe and the testicles of males appeared degenerated with necrotic spermatocytes and spermatozoa present (McConnell et al., 1978b; Vos et al., 1974). Death in mice was frequently attributed to terminal hermorrhages (Vos et al., 1974).

In monkeys exposed to lethal levals of 2,3,7,8-TCDD, McConnell et al. (1978a) reported clinical and histologic signs of toxicity, some of which were similar to those already described for other species. Severe thymic atrophy and edema occurred in treated animals, as well as extensive weight loss that could account for up to 38% of the body mass. As in guinea pigs, liver injury appeared to be mild; however, increased serum GOT and aldolase activity and decreased albumin levels indicative of liver pathology occurred near the time of death. As observed in mice, the bone marrow of monkeys was hypocellular. In addition to the above signs of toxicity, which were observed in other species as well, monkeys had progressive loss of hair, toenails and fingernails, with associated dermatitis consisting of the development of a crusty texture to the skin, squamous metaplasia of sebaceous glands and gastric mucosal dysplasia. As with most other species, a specific cause of death could not be determined for monkeys. Poland and Knutson (1982) summarized the toxic response of various species to 2,3,7,8-TCDD in Table 8-2.

There was very little information on the lethal effects of PCDD congeners other than 2,3,7,8-TCDD. McConnell et al. (1978b) determined the $LD_{50}$ for nine congeners of PCDD following a single treatment by gavage in mice and guinea pigs. A comparison of the $LD_{50}$ expressed as μmol/kg body weight is presented in Table 8-3. The limited data suggest that congeners containing chlorine in the 2,3,7,8 positions were more biologically active than congeners deficient in a chlorine from any one of these positions. It also appears that addition of one or more chlorines to 2,3,7,8-TCDD results in a decrease in lethality. Although the congeners vary in effective dose between mice and guinea pigs, the relative order of toxicity of these congeners did not change. Also, similar effects of toxicity were observed for all congeners as described above for 2,3,7,8-TCDD when the comparison was made within a single species.

## TABLE 8-2

Toxic Responses Following Exposure to 2,3,7,8-TCDD: Species Differences[a]

| | Monkey | Guinea Pig | Cow[b] | Rat | Mouse | Rabbit[b] | Chicken[b] | Hamster |
|---|---|---|---|---|---|---|---|---|
| Hyperplasia and/or metaplasia | | | | | | | | |
| Gastric mucus | ++[c] | 0 | + | 0 | 0 | | | 0 |
| Intestinal mucosa | + | | | | | | | ++ |
| Urinary tract | ++ | ++ | ++ | 0 | 0 | | | |
| Bile duct and/or gall bladder | ++ | 0 | + | | ++ | | | 0 |
| Lung: focal alveolar | | | | ++ | | | | |
| Skin | ++ | 0 | *[d] | 0 | 0 | ++ | | 0 |
| Hypoplasia, Atrophy or Necrosis | | | | | | | | |
| Thymus | + | + | + | + | + | | + | + |
| Bone marrow | + | + | | | ± | | + | |
| Testicle | + | + | | + | + | | + | |
| Other | | | | | | | | |
| Liver lesions | + | ± | | ++ | + | ++ | + | ± |
| Porphyria | 0 | 0 | | + | ++ | | + | 0 |
| Edema | + | 0 | | 0 | + | | ++ | + |

[a] References: monkey (McConnell et al., 1978b; Norback and Allen, 1973; Allen et al., 1977); guinea pig (McConnell et al., 1978b; McConnell, 1980; Moore et al., 1979; Turner and Collins, 1983); cow (McConnell, 1980); rat (McConnell, 1980; Kociba et al., 1978a; Kociba et al., 1979); mouse (Schwetz et al., 1973; McConnell et al., 1978b; Vos et al., 1973); rabbit (Kimmig and Schultz, 1957; Schwetz et al., 1973; Vos and Beems, 1971); chicken (Schwetz et al., 1973; Norback and Allen, 1973; Allen and Lalich, 1962; Vos and Koeman, 1970); hamster (Olson et al., 1980b; Henck et al., 1981).

[b] Responses followed exposure to 2,3,7,8-TCDD or structurally related chlorinated aromatic hydrocarbons.

[c] Symbols: 0, lesion not observed; +, lesion observed (number of "+" denote severity); ±, lesion observed to a very limited extent; blank, no evidence reported in literature.

[d] Skin lesions in cattle are observed, but they differ from the skin lesions observed in other species.

Adapted from Poland and Knutson, 1982.

## TABLE 8-3

### Estimated Single Oral $LD_{50}$ - 30 Values for PCDDs[a]

| Chlorination of PCDDs | Guinea Pigs (μmol/kg)[b] | Mice (μmol/kg)[b] |
|---|---|---|
| 2,8 | >1180 | NR |
| 2,3,7 | 120.41 | >10 |
| 2,3,7,8 | 0.006 | 0.88 |
| 1,2,3,7,8 | 0.009 | 0.94 |
| 1,2,4,7,8 | 3.15 | >14 |
| 1,2,3,4,7,8 | 0.185 | 2.11 |
| 1,2,3,6,7,8 | 0.178-0.255[c] | 3.19 |
| 1,2,3,7,8,9 | 0.153-0.255[c] | >3.67 |
| 1,2,3,4,6,7,8 | >1.400 | NR |

[a] Source: McConnell et al., 1978b

[b] Spearman-Karber method

[c] Estimated range due to variability in replicates

NR = Not reported

8.1.1.2. EFFECTS ON THE LIVER -- The histological and ultrastructural changes in the liver induced by oral exposure to 2,3,7,8-TCDD have been reported by Fowler et al. (1973), Jones and Butler (1974) and Jones (1975). Fowler et al. (1973) treated groups of 30 male rats with a single dose of 2,3,7,8-TCDD at 0.0, 5 and 25 μg/kg by gavage. The animals were killed in groups of 5 on days 1, 3, 6, 9, 16 and 28 after treatment and the livers were prepared for histologic examination. The major ultrastructural change observed was a dose-related increase in the smooth and rough endoplasmic reticulum (ER) in cells near the bile canaliculi. The initial increases appeared at day 3, with the maximal response occurring on days 6 and 9. By day 16 the smooth ER was nearly absent from the parenchymal cells, although large amounts of rough ER were still present. By day 28 the cells had returned to normal appearance. These changes in liver cells following 2,3,7,8-TCDD treatment would be consistent with the induction of protein and RNA synthesis.

Transmission electron microscopic observations revealed that single i.p. administration of 20 μg/kg of 2,3,7,8-TCDD in Sprague-Dawley male rats produces necrotizing hepatic lesions that become progressively worse up to the 16th week postexposure followed by gradual improvement of the condition and disappearance of the lesions (Weber et al., 1983).

At higher doses of 200 μg/kg, Jones and Butler (1974) observed necrosis and proliferative changes in the liver of rats to be the predominant lesions. After treatment by gavage, groups of 4 male and 4 female rats were killed and examined on a weekly basis for 10 weeks. By the first week, degenerating cells were observed near the central vein and these lesions progressed to areas of focal necrosis by the sixth week. Superimposed on the necrotic changes were hyperplasia of the viable cells with multinucleated cells common by the ninth week. At week 10 central vein fibrosis and scattered necrosis remained. Fine structure observed after this large dose of 2,3,7,8-TCDD also revealed increases in smooth ER; however, the most striking effect was degeneration of the plasma membrane with the resulting fusion of parenchymal cells. In a study of similar design, Jones (1975) followed the distribution with time after treatment of membrane associated ATPase activity by histochemical techniques. At 3 days after treatment, the first changes in ATPase patterns were observed, with loss of activity along the canalicular borders and some increased activity in the sinusoids. The midzonal and periportal zones had normal activity at this time. The loss of ATPase activity persisted for 34-42 days and paralleled the histologic lesions described previously (Jones and Butler, 1974). In rats that survived treatment, the ATPase activity was back to normal by 9 months.

Peterson et al. (1979a) further studied the effect of 2,3,7,8-TCDD at lower doses on hepatocyte plasma membrane ATPase activity. Liver surface membranes (LSM) isolated from male Holtzman rats 2, 10, 20 or 40 days after intubation with 2,3,7,8-TCDD at 0.0, 10 or 25 μg/kg were used for determination of $Na^+$, $K^+$-ATPase and $Mg^{++}$-ATPase activity. The activity of $Na^+$, $K^+$-ATPase was depressed to the same extent for both doses of 2,3,7,8-TCDD from day 2-40 after treatment, while a similar depression of the $Mg^{++}$-ATPase activity was observed only in the high dose group. In the low dose group, there was a decrease in $Mg^{++}$-ATPase at 20 days, but recovery to normal levels occurred by 40 days post-treatment. It was demonstrated that the effect of 2,3,7,8-TCDD on ATPase activity was not the result of 2,3,7,8-TCDD induced food deprivation and _in vitro_ studies indicated that the loss of activity was not due to the direct interference of

2,3,7,8-TCDD with the enzyme. Quantitative changes (both increases and decreases) have been reported for the protein composition of plasma membranes isolated and analyzed by electrophoresis from Sprague-Dawley rats 10 days after an i.p. injection of 2,3,7,8-TCDD, indicating that exposure was actually affecting membrane components (Brewster et al., 1982).

Peterson et al. (1979a) observed a positive correlation between the levels of LSM ATPase activity and both in vivo cumulative biliary excretion of ouabain and bile flow (μℓ/min/g liver). Using perfused liver, however, Peterson et al. (1979b) reported a segregation between LSM ATPase activity and biliary excretion of ouabain when 2,3,7,8-TCDD rats were exposed to the protective agents pregnenolone-16α-carbonitrile or spirenolactone. It was concluded that LSM ATPase did not directly participate in ouabain transport.

Additional studies have described the effect of 2,3,7,8-TCDD on the biliary excretion of a variety of xenobiotics. Early studies by Hwang (1973) investigated 2,3,7,8-TCDD inhibition of biliary excretion in male CD rats given a single dose of 2,3,7,8-TCDD at 25 or 5 μg/kg by gavage. Animals were examined for indocyanine green (ICG) excretion 1, 7 and 16 days after treatment. Unlike Peterson et al. (1979a), Hwang (1973) observed an inverse relationship between 2,3,7,8-TCDD exposure and bile flow, with maximum bile flow observed in the 25 μg/kg dose group at 16 days. Even with this increased bile flow, however, the cumulative biliary excretion of ICG was decreased in a dose-dependent manner with the greatest depression observed 7 and 16 days after the exposure to 2,3,7,8-TCDD. The levels of ICG in the plasma and liver was higher in treated animals than in control animals, while the concentration in the bile was lower, reflecting the decrease in total excretion of ICG.

Yang and Peterson (1977) compared the effect of 2,3,7,8-TCDD on the biliary excretion of the organic neutral compound, ouabain, with that of the organic anions phenol-3,6-dibromophthalein (DBSP) and sulfobromophthalein (BSP) in male Holtzman rats. Animals were intubated with 2,3,7,8-TCDD at doses of 10 or 25 µg/kg and excretion was evaluated periodically between 2 and 4 days postexposure. The biliary excretion of ouabain was depressed in a dose-related manner starting on the second day post-treatment, with maximum depression developing between 10 and 20 days, and some recovery observed by day 40. Decreases in bile flow followed a pattern similar to that observed for ouabain. The pattern of biliary excretion was different for DBSP and BSP in which only a transient small decrease was observed 10 days after exposure in the high-dose group. In the low-dose animals there was actually an increase at days 10 and 25 in the excretion of the anions. The results obtained for DBSP and BSP differ sharply from those for the organic neutral ouabain or those reported by Hwang (1973) for the organic anion ICG, in which a dose-related decrease in biliary excretion was observed. The authors concluded that the effects of 2,3,7,8-TCDD on the multiple pathways involved in biliary excretion depend on the specific compound being studied.

In the guinea pig and rhesus monkey, which develop little liver pathology after exposure to 2,3,7,8-TCDD, there was also little change in ICG blood clearance rates; in the rabbit, which develops 2,3,7,8-TCDD-induced liver damage similar to the rat, there was reduced blood clearance of ICG (Seefeld et al., 1979, 1980). In the rabbit, there were increases in serum sorbitol dehydrogenase and glutamic pyruvic transaminase activity as further indications of 2,3,7,8-TCDD-produced liver damage. In the monkey, which received 2,3,7,8-TCDD by gavage at doses of 5, 25 or 75 µg/kg, there was an initial slight increase in the blood clearance of ICG at 2 days post-treatment, followed in the two higher-dose groups by a dramatic decrease a few days before death. Although some serum enzymes (sorbitol dehydrogenase and glutamic pyruvic transaminase) indicative of liver damage were elevated, the histopathology of the liver was within normal limits. It appears that major effects on biliary excretion occur only in species that are sensitive to the hepatotoxic effects of 2,3,7,8-TCDD.

Other gross signs of the hepatotoxic effects of 2,3,7,8-TCDD observed in some species included fatty degeneration and porphyria. Early observations by Cunningham and Williams (1972) described a decrease in _in vivo_ (1 hour pulse) incorporation of $^3$H sodium acetate into liver lipids after exposure of male Wistar rats to 2,3,7,8-TCDD. The rats (12-16 animals) were treated with 2,3,7,8-TCDD at a dose of 10 μg/kg followed in either 3 or 7 days by the assessment of lipid synthesis. At 3 days incorporation decreased from 258 to 98 dpm/mg lipid in the control and treated animals, respectively. There was an approximately similar decrease observed 7 days postexposure. When individual classes of lipids were examined, there was a decrease in the synthesis of triglycerides, diglycerides and phospholipids. Although Cunningham and Williams (1972) observed that 2,3,7,8-TCDD decreased lipid synthesis, Albro et al. (1978) reported an increase in total lipids in the livers of rats 13 days after treatment with 2,3,7,8-TCDD at a lethal dose of 50 μg/kg. For individual classes of lipids there was an increase in free fatty acids and cholesterol esters; no change occurred in the content of phospholipids, free cholesterol or triglycerides. The fatty changes in the liver were confirmed by ultrastructural examination of liver specimens. At a sublethal dose of 10 μg/kg there was a different pattern of lipid accumulation; triglycerides and fatty acids increased and cholesterol esters decreased. The changes in the lipid profile of the liver was attributed to

2,3,7,8-TCDD induced mobilization of body fat, a decrease in lysosomal acid lipase (74% decline in this enzyme 10 days after a 50 μg/kg dose of 2,3,7,8-TCDD) and an increase in lipid peroxidation as indicated by a sharp increase in the production of lipofuscin pigments.

Porphyria was initially characterized quantitatively in mice by Goldstein et al. (1978). Groups of 12 male C57Bl mice received 4 weekly intubations of 2,3,7,8-TCDD at doses of 0.0, 1, 5 or 25 μg/kg, or a single dose of 150 μg/kg followed 21-25 days after treatment by analysis of the liver for porphyrins. Porphyrin levels were unchanged except in the 25 and 150 μg/kg groups where the levels were increased 2000- and 4000-fold, respectively. The difference in responsiveness to the development of porphyria was studied by Smith et al. (1981) in C57Bl mice that were sensitive to, and DBA/2 mice that were insensitive to, the toxicity of 2,3,7,8-TCDD. Male and female C57Bl mice had a dose-related increase in hepatic porphyrins in the two high dose groups 3 weeks after a single exposure to 2,3,7,8-TCDD at 0.0, 5, 15, 50 or 75 μg/kg; however, only minimal nondose-related changes in hepatic porphyrin were observed in DBA/2 mice exposed to up to 1200 μg/kg. In the sensitive C57Bl mice there was only a small difference in hepatic porphyrin between the sexes even though males were >3 times as sensitive to the toxic effects of 2,3,7,8-TCDD than females (see Table 8-1). Results similar to those above were reported for urinary porphyrin levels in male C57Bl and DBA/2 mice given 6 weekly doses of 2,3,7,8-TCDD at 25 μg/kg (Jones and Sweeney, 1980). In the sensitive strain, the initial elevation of porphyrin occurred in the second week.

In rats increased urinary porphyrin was observed only after subchronic exposure to 2,3,7,8-TCDD (Cantoni et al., 1981). Female CD rats were administered weekly oral doses of 2,3,7,8-TCDD at levels of 0.01, 0.1 and 1.0 μg/kg for 45 weeks. The initial increase was observed in the high-dose group at 3 months, and in the other two groups at 4 months, after the start of exposure. Not only did the absolute amount of porphyrin increase, but the relative distribution also changed to compounds containing more carboxyl groups. Only in the high dose group did the livers, at the terminal necropsy, show signs of excess porphyrin under examination by UV light.

In attempts to understand the mechanism of 2,3,7,8-TCDD induced porphyria, the effects of 2,3,7,8-TCDD on the enzymes involved in the synthesis and catabolism of porphyrin have been studied. Goldstein et al. (1978) showed that δ-aminolevulinic acid synthetase, a rate-limiting enzyme in porphyrin synthesis, was slightly increased (2-fold) in male C57Bl mice given 4 weekly doses of 2,3,7,8-TCDD at 25 μg/kg. This dose of 2,3,7,8-TCDD increased liver porphyrin levels 2000-fold. Catabolism of porphyrin by uroporphyrinogen decarboxylase (UD) also appeared to be decreased in 2,3,7,8-TCDD treated mice. Smith et al. (1981) reported a decrease in UD activity from ~25 to 7 n moles/hr/g liver in male and female C57Bl mice 3 weeks after a single oral exposure to 2,3,7,8-TCDD at a dose of 75 μg/kg. No effect of 2,3,7,8-TCDD on UD activity was observed in DBA/2 mice that were insensitive to the induction of porphyria. A time course of changes in UD activity with length of time after exposure to 2,3,7,8-TCDD indicated a steady decline in activity starting 3 days after exposure to 2,3,7,8-TCDD, which continued until day 21 when the study was terminated. Sweeney and Jones (1978) reported similar results after 5 weekly doses of 2,3,7,8-TCDD at 25 μg/kg. In this study the UD activity declined ~48% in C57Bl mice and only 4% in DBA/2 mice. Other factors besides the increase in δ-aminolevulinic acid synthetase and the decrease in UD activity may also participate in the dramatic increase in liver porphyrin in mice associated with exposure to near lethal doses of 2,3,7,8-TCDD.

As a result of the protracted time observed between exposure to 2,3,7,8-TCDD and the development of toxic effects, as well as the reported teratogenic and carcinogenic potential of 2,3,7,8-TCDD, investigations have been conducted to determine the influence of 2,3,7,8-TCDD on DNA synthesis in the liver. Greig et al. (1974) measured the in vivo incorporation of $^3$H-thymidine (1 hour pulse) into liver DNA of male and female Porten strain rats after a single exposure to 2,3,7,8-TCDD at doses of 10 and 200 μg/kg. When the 2,3,7,8-TCDD was given either 0, 24 or 72 hours before a 3/4 partial hepatectomy there was only a slight, but not significant, decrease in thymidine incorporation observed when DNA synthesis was measured 24 hours after the operation.

Although 2,3,7,8-TCDD had no effect on in vivo DNA synthesis, similar studies by Conway and Matsumura (1975) and Dickens et al. (1981) demonstrated an increase in thymidine incorporation when determined in vitro. Conway and Matsumura (1975) administered male Sprague-Dawley rats 2,3,7,8-TCDD at a dose of 5 μg/kg followed in 10 days by removal of the liver and the in vitro determination of DNA synthesis in liver slices. Incorporation of thymidine into the nuclei increased from 29 cpm/mg in control animals to 45 cpm/mg in treated animals. A similar near doubling of DNA synthesis was observed by Dickens et al. (1981); however, when DNA synthesis was stimulated by a 1/3 partial hepatectomy, thymidine incorporation into liver slices was increased 10-fold in rats treated 5 days earlier with 2,3,7,8-TCDD as compared with hepatectomized controls. The onset of DNA synthesis after partial hepatectomy (~20 hours) was the same in both 2,3,7,8-TCDD treated and control animals; however, the treated animals had a more rapid and extensive increase in DNA synthesis between 20 and 32 hours after the partial hepatectomy. The rates of DNA synthesis were again the same in both groups 35 hours after the operation. It was shown by hydroxyurea inhibition that the DNA synthesis in both the treated and control animals was predominantly semiconservative. Further studies are needed to determine the reason for the difference observed between in vitro and in vivo measurements of DNA synthesis in the liver after exposure to 2,3,7,8-TCDD.

Extensive hepatic necrosis in the rabbit may be responsible for death in this species (Poland and Knutson, 1982).

Besides the effects on the liver of 2,3,7,8-TCDD exposure described above, it is known that 2,3,7,8-TCDD is a potent inducer of microsomal enzymes. These studies will be discussed in Section 8.1.1.5., which describes the ability of this xenobiotic to induce microsomal enzymes in a number of tissues and organs.

8.1.1.3. EFFECTS ON OTHER ORGAN SYSTEMS -- The most noticeable feature of 2,3,7,8-TCDD toxicity is the loss of body weight and the apparent "wasting away" until death. Since decreased food consumption may not totally account for these findings, the effect of 2,3,7,8-TCDD on intestinal absorption has been studied. Madge (1977) assessed the ability of the intestine to absorb D-glucose, D-galactose, L-arginine and L-histidine using the everted intestinal sac technique in CD-1 mice exposed to 2,3,7,8-TCDD. In measurements made 7 days after treatment with doses of 0.0, 10, 25, 75, 150, 200 or 300 μg/kg, D-glucose was absorbed to a lesser degree at all doses than in control animals. The two low doses produced a dose-related decrease in absorption; however, at doses of $\geq$75 μg/kg the decrease was uniform. At a dose of 150 μg/kg, decreased absorption of D-glucose was slight 3 days after treatment, became maximally decreased by 7 days, and this depressed level was maintained for 28 days, at which time the study was terminated. Providing D-mannose to the incubation mixture as an energy supply increased the absorption of D-glucose to control levels; however, the amount of D-glucose on the serosal side was still lower than control levels. This suggested that intestinal utilization of D-glucose was taking place and might account for some of the observed malabsorption. Treatment with 2,3,7,8-TCDD had no effect on the absorption of the other compounds investigated. In a similar experiment in Sprague-Dawley rats, Ball and Chhabra (1981) also observed malabsorption of D-glucose. In this study, however, absorption of leucine was also decreased. The decrease in leucine absorption took longer to manifest itself; a significant decrease was observed only after 2 weeks treatment with 2,3,7,8-TCDD.

In contrast to the results observed for D-glucose, intestinal iron transport was shown to be elevated by exposure to 2,3,7,8-TCDD. Manis and Kim (1979a) examined the effect of prior treatment of male Sprague-Dawley rats on the 30-minute transport of $^{59}$Fe out of a duodenal loop created by ligating a section of the intestine _in situ_. At single 2,3,7,8-TCDD doses of between 22 and 84 μg/kg there was increased serosal transfer of $^{59}$Fe measured 48 hours after treatment. At doses >42 μg/kg the increase was ~100%. The time after treatment at which serosol transfer was greatest was 1 day, with rapid decline in stimulation to near the levels of controls observed on days 2-7. There was also an apparent effect of route of administration, with gavage treatment being more effective in inducing iron transport than i.p. injection. In similar experiments calcium transport was decreased, and glulactose and proline transport were unaffected by prior exposure to 2,3,7,8-TCDD. Manis and Kim (1979b) had identical results when the everted intestinal sac was used to assess iron transport. It was interesting to note that only duodenal sacs were stimulated, with no effect of 2,3,7,8-TCDD exposure observed in the adjacent distal segment of the intestine. Increased iron transport was also observed by Manis and Kim (1979a) in an unidentified strain of mice. Increased iron transport may be one of the earliest effects of 2,3,7,8-TCDD; however, at present the toxicologic relevance of this transient disturbance in iron transport is unknown.

One of the common gross observations of 2,3,7,8-TCDD toxicity is severe edema, suggestive of a breakdown in salt and water homeostasis. These observations prompted investigations to determine the effect of 2,3,7,8-TCDD on the function of the kidney. Pegg et al. (1976) measured renal function in vitro using renal cortical slices obtained from male Sprague-Dawley rats 3 and 7 days after intubation with 2,3,7,8-TCDD at doses of 10 or 25 µg/kg. (These results were also described by Hook et al., 1977). Anion and cation transport were measured by the respective accumulation of p-aminohippuric acid and N-methylnicotinamide into the cortical slices. Anion accumulation was lower in the high dose group; cation transport was lower at both dose levels tested. The decrease in anion transport was confirmed in an in vivo study. Ammoniogenesis and gluconeogenesis were not affected in 2,3,7,8-TCDD treated rats, even when the animals were made acidotic, which suggests no effect on the kidneys' ability to maintain acid base balance. Also, sodium reabsorption was shown in vivo to be within normal range. Since decreases in cation and anion transport were the only effects observed, and since these compounds are transported by a different mechanism, the authors concluded that the effect of 2,3,7,8-TCDD was merely a general decrease in kidney functon reflecting the poor condition of the treated animals (animals in all treated groups had decreased weight gain), and not a cause of debilitation.

Although kidney function was only minimally affected by exposure to 2,3,7,8-TCDD, Greig et al. (1974) demonstrated that pre-exposure to 2,3,7,8-TCDD could reduce the ability of the rat kidney to respond to stimuli of DNA sythesis. Folate-stimulated DNA synthesis measured in vivo in Porten strain rats was decreased between 67 and 25% in animals receiving 2,3,7,8-TCDD at a dose of 10 μg/kg on day 0-9 before administration of folic acid. No significant difference in folate-stimulated DNA synthesis was observed if 2,3,7,8-TCDD was given 23 hours after folic acid. The lack of effectiveness of administering 2,3,7,8-TCDD shortly after treatment with folic acid suggested that 2,3,7,8-TCDD did not directly interact with cellular DNA, nor inhibit the protein synthesis necessary to support folate-stimulated DNA synthesis. Similar inhibitory effects of 2,3,7,8-TCDD were observed when lead acetate was used to stimulate kidney DNA synthesis. The mechanism by which 2,3,7,8-TCDD prevents the kidney from responding to proliferative stimuli is not known, although it was demonstrated that another agent capable of inducing microsomal enzymes, 3-methylcholanthrene (3-MC), had similar effects on the kidney.

Additionally a number a hematologic and clinical chemistry changes have been observed in the blood of laboratory animals after exposure to 2,3,7,8-TCDD. Many of these changes, as described by Zinkl et al. (1973), reflect damage to previously described organ systems. In female CD rats given 30 daily doses of 2,3,7,8-TCDD at levels of 0.1, 1.0 or 10 μg/kg, the clinical chemistry of the serum reflected liver damage. In the high-dose group, serum GOT and serum GPT were elevated starting 13-17 days after initial treatment. There was a marginal change in GPT in the mid-dose group and lactic dehydrogenase (LDH) in the high-dose group, but the increases were only transitory. Serum cholesterol was increased in the high-dose animals starting at day 10, with a transitory increase again observed in the mid-dose group. Conversely, there was a decrease in serum protein from day 24 on in the high-dose animals. Along with these clinical chemistry changes indicative of liver damage, the only other major effect observed in the blood was thrombocytopenia. The decrease in platelet count was detected early, by day 3, in the 10 and 1 μg/kg groups; in the 0.1 μg/kg group a significant decrease was not observed until day 17. Thrombocytopenia was also observed in female guinea pigs after 8 weekly oral doses of 2,3,7,8-TCDD at 0.2 μg/kg, and in mice (administered a single dose of 1.0, 10 or 50 μg/kg). In guinea pigs lymphopenia was also observed. Other hematologic changes were attributed to hemoconcentration.

In a more extensive investigation of 2,3,7,8-TCDD-induced hyperlipidemia in male Sprague-Dawley rats, Poli et al. (1980) treated animals with a single i.p. injection of 2,3,7,8-TCDD at 2 doses of 2.5, 5, 10 and 20 μg/kg. At day 21 after treatment there was a dose-related increase in total plasma cholesterol and high density lipoprotein cholesterol, while no change was observed in triglycerides or very low and low density lipoproteins (VLDL and LDL, respectively). At a dose of 20 μg/kg the maximum increase in HDL cholesterol and total cholesterol occurred 30 days after treatment, and a significant elevation was still present at 60 days after treatment when the study was terminated. Slight changes in the apoprotein of HDL from 2,3,7,8-TCDD rats and control rats were indicative of new apoprotein synthesis. Although the increases in HDL cholesterol may be in response to eliminating excess lipids, the exact function has not been clearly shown. There is some evidence from studies of workers exposed to 2,3,7,8-TCDD that there were reduced levels of blood HDL cholesterol and raised total cholesterol as compared with a matched control group (Walker and Martin, 1979).

In contrast to rats, male Hartley strain guinea pigs given a single i.p. injection of 2,3,7,8-TCDD at a dose of 2 μg/kg had increased hyperlipidemia characterized by increases in VLDL and LDL (Swift et al., 1981). In animals sacrificed 7 days after exposure to 2,3,7,8-TCDD, there was an increase in total serum lipid, cholesterol esters, triglycerides and phospholipids, when comparison was made with pair-fed, weight-paired or _ad libitum_ fed control groups. Serum-free fatty acids were not changed quantitatively; however, some qualitative changes occurred, reflecting an increase in the types of fatty acids that were abundant in the adipose tissue of guinea pigs. Anaylsis of lipoproteins revealed a 19-fold increase in VLDL, a 4-fold increase in LDL, and no change observed in the levels of HDL. The VLDL was also qualitatively different in the 2,3,7,8-TCDD treated animals, containing less cholesterol ester and an altered C apoprotein. The importance of these qualitative changes is unclear. The hyperlipidemia may result from the 2,3,7,8-TCDD mobilization of free fatty acids, which are then used in the synthesis of VLDL and are subsequently formed into LDL. The relationship of the changes in serum lipid levels to the mechanism of 2,3,7,8-TCDD toxicity needs further study.

Elovaara et al. (1977) observed some changes in biochemicals of the brain of male Wistar and heterozygous Gunn rats given a single intubation of 2,3,7,8-TCDD at a dose of 20 μg/kg. At 7 days post-treatment, there was a small but significant decrease as compared with vehicle treated control animals in both the protein and RNA content of the Wistar rats, while levels of acid proteinase and DT-diaphorase (an enzyme induced by 2,3,7,8-TCDD in the liver) had a small but significant increase in the heterozygous Gunn rats. There were no significant changes observed in homozygous rats given 2,3,7,8-TCDD at 20 µg/kg. The authors noted that acid proteinase may participate in chemically induced degeneration of the brain.

8.1.1.4. IMMUNOLOGICAL EFFECTS -- During acute toxicity studies with 2,3,7,8-TCDD, thymic atrophy was noted as a consistent effect in all species that have been investigated. This finding suggested that 2,3,7,8-TCDD may alter the immune response, and initiated immunotoxicity studies in exposed animals. In guinea pigs treated with 8 weekly oral doses of 2,3,7,8-TCDD (0, 0.008, 0.04, 0.2 or 1.0 µg/kg bw), body weight, spleen weight and thymus weight were depressed, adrenal weight was increased and leukocyte and lymphocyte counts were elevated (Vos et al., 1973). Upon histological examination, 2,3,7,8-TCDD-exposed rats had a severe depletion of lymphocytes from the thymic cortex (Vos and Moore, 1974). Hematological changes were noted in rats exposed to 10 and 14 daily doses of 10 µg/kg 2,3,7,8-TCDD (Weissberg and Zinkl, 1973). Increased red blood cell count, decreased platelet count, increased neutrophil count and increased packed cell volumes were reported in 2,3,7,8-TCDD-exposed rats. A summary of the data available on the immunotoxic effects of 2,3,7,8-TCDD in animals is presented in Table 8-4. A review of immunotoxicity and immunosuppression was reported by Vos (1977).

Vos et al. (1973) investigated the humoral and cell-mediated immune response in Hartley guinea pigs, CD rats and B6D2F1 mice. The humoral immune response was tested in 2,3,7,8-TCDD-treated hamsters by injecting tetanus toxoid (subcutaneously) into the footpad and later testing for the concentration of tetanus antitoxin from the serum by an immunodiffusion

TABLE 8-4

Immunological Effects of 2,3,7,8-TCDD in Animals

| Species/ Strain | Sex | Exposure Route | Dose(s) | Duration of Exposure | Minimum Effective Dose | Parameter | Effect | Reference |
|---|---|---|---|---|---|---|---|---|
| Mice/B6D2F1 | M | gavage | 0, 0.2, 1.0, 5.0, 25.0 μg/kg bw/week | 4 weeks | NA | bw | no change | Vos et al., 1973 |
| | | | | | 5.0 μg/kg bw/week | thymus weight | decreased | |
| | | | | | 5.0 μg/kg bw/week | graft-versus-host response | decreased | |
| Mice/C57Bl/6 | F,M | maternally administered (gavage) | 0, 1.0, 2.0, 5.0, 25.0 μg/kg | 4 or 6 weeks (3 or 5 administrations) | 1.0 μg/kg bw/week | thymus weight | decreased | Vos and Moore, 1974 |
| | | | | | 25.0 μg/kg bw/week | PHA response | decreased | |
| | | | | | 2.0 μg/kg bw/week | skin graft rejection | prolonged | |
| Mice/ C57Bl/6Jfh | M | gavage | 0, 0.5, 1, 5, 10, 20 μg/kg bw/week | 4 weeks | 1.0 μg/kg bw/week | _Salmonella_ infection | increased mortality and decreased time to death | Thigpen et al., 1975 |
| Mice/Swiss | M | gavage | 0, 1.5, 5, 15, 50 μg/kg bw/week | 4 weeks | 1.5 μg/kg bw/week | endotoxin (_E. coli_) susceptibility | increased mortality | Vos et al., 1978a |
| Mice/B6C3F1 | F | _in vitro_ (spleen cells) | 0.5, 5.0, 50 ng/mℓ | 5-60 seconds | 50 μg/mℓ | protein, DNA, and RNA synthesis | decreased | Luster et al., 1979a,b |
| Mice/Swiss-Webster | F,M | maternally administered (diet) | 0, 1, 2.5, 5, 10, 20 ppb (dietary) | 10 weeks (pregestation and 3 weeks post-parturition) | 2.5 ppb | antigenic RBC reaction | decreased | Thomas and Hinsdill, 1979 |
| | | | | | 2.5 ppb | thymic cortex | atrophy | |
| | | | | | 5 ppb | contact sensitivity to DNFB | decreased | |
| | | | | | 1 ppb | endotoxin (_Salmonella_) susceptibility | increased mortality | |
| | | | | | NA | _Listeria_ infection | no change | |
| Mice/CD | M | gavage | 0, 0.01, 0.1, 1.0, 10.0 μg/kg bw/week | up to 8 weeks | 0.01 μg/kg bw/week | serum immunoglobin level | increased | Sharma and Gehring, 1979 |
| | | | | | 1.0 μg/kg bw/week | serum immunoglobin level | decreased | |
| Mice/CD | M | _in vitro_ | $10^{-4}$-$10^{-8}$ M | single | $10^{-8}$ M | lymphocyte blastogenic transformation | increased | Sharma and Gehring, 1979 |

TABLE 8-4 (cont.)

| Species/ Strain | Sex | Exposure Route | Dose(s) | Duration of Exposure | Minimum Effective Dose | Parameter | Effect | Reference |
|---|---|---|---|---|---|---|---|---|
| Mice/Swiss-Webster | F | oral (diet) | 0, 10, 100 ppb | 5 weeks (or more) | 10 ppb | tetanus response | decreased | Hinsdill, et al., 1980 |
| | | | | | 10 ppb | antigenic RBC response | decreased | |
| | | | | | 10 ppb | sensitization to DNFB | decreased | |
| | | | | | 10 ppb | resistance to *Salmonella* | increased mortality | |
| | | | | | 10 ppb | resistance in *Listeria* | increased mortality | |
| Mice/ C57Bl/6J | M | i.p. | 0, 1, 2, 6, 30 µg/kg bw | single injection | 1 µg/kg | macrophage and natural killer cell activity | no change | Mantovani et al., 1980 |
| | | | | | 1 µg/kg | macrophage and natural killer cell number | decreased | |
| | | | | | | antibody production | decreased | |
| Mice/B6C3F1 | M,F | maternally administered | 0, 1.0, 5.0, 15.0 µg/kg bw/day | 4 days during gestation and lactation | 1.0 µg/kg bw/day | *L. monocytogenes* susceptibility | increased | Luster et al., 1980 |
| | | | | | 1.0 µg/kg bw/day | PYB6-tumor susceptibility | increased | |
| | | | | | 5.0 µg/kg bw/day | bone marrow hypocellularity | increased | |
| Mice/C57Bl/6 | M | i.p. | 0, 0.4, 4.0, 40 µg/kg bw/week | 4 weeks | 4.0 µg/kg bw/week | thymus atrophy | increased | Clark et al., 1981 |
| | | | | | 0.4 µg/kg bw/week | cytotoxic T-cell response | decreased | |
| Mice/C57Bl/6 | M | i.p. | 0, 0.004, 0.04, 0.4 µg/kg bw/week | 4 weeks | 0.004 µg/kg bw/week | *in vitro* generation of cytotoxic T-cells | decreased | Clark et al., 1981 |
| Rat/CD | F | oral | 0, 0.2, 1.0, 5.0 µg/kg bw/week | 6 weeks | 5.0 µg/kg bw/week | bw | decreased | Vos et al., 1973 |
| | | | | | 5.0 µg/kg bw/week | thymus weight | decreased | |
| | | | | | NA | tuberculin hypersensitivity | no change | |
| Rat/CD | F | oral | 0, 10 µg/kg bw/day | 10, 14 days | 10 µg/kg bw/day | erythrocyte count | increased | Weissberg and Zinkl, 1973 |
| | | | | | 10 µg/kg bw/day | platelet count | decreased | |
| | | | | | 10 µg/kg bw/day | neutrophil count | increased | |

TABLE 8-4 (cont.)

| Species/ Strain | Sex | Exposure Route | Dose(s) | Duration of Exposure | Minimum Effective Dose | Parameter | Effect | Reference |
|---|---|---|---|---|---|---|---|---|
| Rat/F-344 | F,M | maternally administered | 0, 1.0, 5.0 μg/kg bw/dose | 4 or 6 weeks (3 or 5 administrations) | 1.0 μg/kg bw/dose | bw and thymus weight | decreased | Vos and Moore, 1974 |
| | | | | | 5.0 μg/kg bw/dose | spleen weight | decreased | |
| | | | | | 5.0 μg/kg bw/dose | PHA response | decreased | |
| | | | | | 5.0 μg/kg bw/dose | graft-versus-host response | decreased | |
| | | | | | 5.0 μg/kg bw/dose | skin graft rejection | prolonged | |
| | | | | | NA | pseudorabies virus infection | no change | |
| Rat/Fischer | F,M | maternally administered (NR) | NR | 4-6 weeks (during gestation and neonatally) | NR | Con A and PHA response | decreased | Moore and Faith, 1976 |
| | | | | | NR | oxazolone skin hypersensitivity | decreased | |
| Rat/Fischer-Wistar | F,M | maternally administered (NR) | 0, 5 μg/kg bw/dose | 3 or 4 applications during gestation and neonatally | 5 μg/kg bw/dose | antibody production to bovine gamma globulin | no effect | Faith and Luster, 1979 |
| | | | | | 5 μg/kg bw/dose | PHA and Con A response | decreased | |
| | | | | | 5 μg/kg bw/dose | thymus and bw | decreased until 128 days | |
| Rat/Sprague-Dawley | M | i.v. | 0, 1 μg/kg bw | single injection | 1 μg/kg bw | thymic RNA synthesis | decreased | Kurl et al., 1982 |
| | | | | | | thymic RNA polymerase activity | decreased | |
| Guinea pig/ Hartley | F | gavage | 0, 0.008, 0.04, 0.2, 1.0 μg/kg bw | 8 weeks | 0.04 μg/kg bw/week | bw | decreased | Vos et al., 1973 |
| | | | | | 0.04 μg/kg bw/week | thymus weight | decreased | |
| | | | | | 0.04 μg/kg bw/week | tuberculin hypersensitivity | decreased | |
| | | | | | 0.2 μg/kg bw/week | tetanus antitoxin | decreased | |

M = male; F = female; i.p. = intraperitoneal i.v. = intravenous; PHA = Phytohemagglutinin; Con A = Conconavalin A; RBC = red blood cell; DNFB = 2,4-dinitro, 1-fluorobenzene; NA = Not applicable; NR = Not reported technique. Cell-mediated immunity was tested by injecting Mycobacterium tuberculosis (subcutaneously) into guinea pigs on day 35 of 2,3,7,8-TCDD treatment (during a schedule of 8 weekly doses). Intradermal tuberculin hypersensitivity was determined by measurements of skin thickening on days 47 and 54. Decreased skin hypersensitivity was noted in hamsters treated with 0.04 g 2,3,7,8-TCDD/kg and higher doses. Decreased tetanus antitoxin levels were evident in guinea pigs treated with 0.2 µg 2,3,7,8-TCDD/kg, but not at lower dose levels. Vos et al. (1973) also tested the cell-mediated immunity in rats exposed to 2,3,7,8-TCDD (0, 0.2, 1.0 or 5.0 µg/kg, once weekly for 6 weeks). M. tuberculosis was injected into rats by day 28 of the treatment period, followed by intradermal hypersensitivity testing on day 42. No changes in the thickness of skin were noted in 2,3,7,8-TCDD treated rats when compared with controls.

Mice were used to test the effect of 2,3,7,8-TCDD on cell-mediated immunity by use of the "graft-versus-host" experiment (Vos et al., 1973). In this test, spleen cells from 2,3,7,8-TCDD-exposed mice (0, 0.2, 1.0 or 5.0 µg/kg once weekly for 4 weeks) of the C57B1/6 strain were injected into the right footpad of a hybrid recipient mouse (C57B1/6 x DBA-2). Donor cells possessing sufficient activity will respond to the DBA-2 antigen on the host cells, resulting in the enlargement of the popliteal lymph node. Host cells are tolerant of the donor cells since both have C57B1/6 antigens. In this test Vos et al. (1973) noted a significant ($p<0.01$) dose-related decrease in the activity of 2,3,7,8-TCDD-treated spleen cells (as measured by the degree of popliteal lymph node enlargement on the site of the spleen cell injection). Lymph node enlargement was significantly less ($p<0.01$) in hybrid recipient mice receiving spleen cells from mice treated with 5 µg 2,3,7,8-TCDD/kg/week than from donor cells of untreated mice.

Studies continued in an attempt to identify the mechanism of 2,3,7,8-TCDD-induced immunodeficiency. Rats (F-344) exposed pre- and postnatally by maternal dosing (1 or 5 μg 2,3,7,8-TCDD/kg administered to dams on days 11 and 18 of gestation and 0, 7 and 14 postnatally) had prolonged times until graft rejection, decreased spleen cell graft-versus-host activity and decreased binding response to phytohemagglutinin (PHA) (Vos and Moore, 1974; Moore and Vos, 1974). Response to conconavalin A (Con A), a humoral immune response, was actually increased.

Since thymus-derived lymphocytes (T-cells) play a central role in cell-mediated immunity and host defense mechanisms, interest turned to these areas of immunology. The effect of 2,3,7,8-TCDD on host resistance to infection, a vital measure of immune response, was tested by Thigpen et al. (1975) in male pathogen-free mice (C57Bl/6Jfh). 2,3,7,8-TCDD was administered to mice at 0.5, 1, 5, 10 or 20 μg/kg once weekly for 4 weeks followed by inoculation with Salmonella bern 2 days after the final 2,3,7,8-TCDD administration. Mortality rates and "time until infection" were used to determine the immunological effect of 2,3,7,8-TCDD. A significant ($p<0.05$) increase in mortality and decrease in time of infection were noted in groups treated with 1 μg/kg or higher doses of 2,3,7,8-TCDD when compared with controls. 2,3,7,8-TCDD at 0.5 μg/kg did not alter these parameters and was regarded as a no effect level. The immune-resistance of mice to S. bern is therefore reduced by treatment with 1 μg 2,3,7,8-TCDD/kg/week (for 4 weeks).

Pretreatment with 2,3,7,8-TCDD greatly enhances the susceptibility of mice to E. coli endotoxin (Vos et al., 1978a). Injection of 250 μg of endotoxin to mice pretreated with 0, 1.5, 5 and 15 μg 2,3,7,8-TCDD/kg resulted in 0/5, 1/5, 6/6 and 6/6 deaths, respectively. Mice pretreated with 15 and 50 μg 2,3,7,8-TCDD/kg and injected with 10 μg of endotoxin had 1/4 and 2/4 deaths, respectively. Mice treated with lower doses of 2,3,7,8-TCDD were not susceptible to this quantity of endotoxin. Increased mortality (2/6) in a control group was noted only when 500 μg of endotoxin was administered; however, 10 μg of endotoxin was sufficient to cause similar mortality (2/5) in mice treated with 50 μg 2,3,7,8-TCDD/kg.

The immunocompetence of 5-week-old offspring of Swiss-Webster mice fed diets containing 1, 2.5, 5, 10 or 20 ppb 2,3,7,8-TCDD was tested by several means (Thomas and Hinsdill, 1979). The number of cells reactive to antigenic RBC, differential white blood cell counts, organ weights, histopathologies, hypersensitivity to 2,4-dinitro-1-fluorobenzene (DNFB) and the resistance to *E. coli* lipopolysaccharide (LPS), *Listeria monocytogenes* and *Salmonella typhimurium* LPS were all measured for mice exposed to different levels of 2,3,7,8-TCDD. Adult female mice were exposed to 2,3,7,8-TCDD for 4 weeks before mating, throughout gestation and for 3 weeks postparturition. Young mice being tested for immunotoxicity were therefore exposed to 2,3,7,8-TCDD only *in utero* and through lactation. The typical decrease in thymus weight was noted in mice exposed to 2.5 and 5.0 ppb but was not evident in the 1.0 ppb group. A decrease in the number of plaque-forming cells (PFC) reactive to sheep RBCs was significantly reduced in the 2.5 and 5.0 ppb 2,3,7,8-TCDD-exposed groups. (Because of the poor survival of young in the 10 and 20 ppb 2,3,7,8-TCDD-exposed groups, results and comparisons were usually reported for the three lower dose groups). The humoral content of anti-RCD antibodies, however, was not lower in 2,3,7,8-TCDD-exposed groups when compared with controls. A decrease in the skin hypersensitivity to DNFB following sensitization was noted in all 2,3,7,8-TCDD-treated groups (only the 5-ppb group was statistically reduced from controls). 2,3,7,8-TCDD caused an increased susceptibility (increased mortality level) to S. typhimurium in a dose-related fashion. The response to E. coli LPS and L. monocytogenes was not different from controls. 2,3,7,8-TCDD exposure did not alter the response of lymphocytes (Band T-cells) in vitro to Con A, nor was mitogen-induced lymphocyte proliferation affected (Thomas and Hinsdill, 1979).

Similar findings were reported in Fischer/Wistar rats exposed to 2,3,7,8-TCDD during gestation (18th day) and neonatally, or neonatally alone (on days 0, 7 and 14) (Faith and Luster, 1979). Dams were treated with 5 g/kg 2,3,7,8-TCDD on each dose day. Typically, body weight and thymic weights were decreased in progeny, which lasted until 135 days of age. The thymic- and splenic-cell response to PHA and Con A was decreased in all 2,3,7,8-TCDD-treated animals and did not return to normal until day 270. Delayed hypersensitive reaction was also suppressed until 270 days of age. The production of antibodies to bovine gamma globulin, which requires T-helper cell function, was not affected by 2,3,7,8-TCDD exposure during rat development (Faith and Luster, 1979).

Neonatal B6C3F1 mice, exposed to prenatal (maternal dosing on day 14 of gestation) and postnatal (days 1, 7 and 14 after birth) doses of 0, 1.0, 5.0 or 15.0 μg/kg 2,3,7,8-TCDD, were studied for immunotoxic effects and host susceptibility (Luster et al., 1980). At the 15.0 μg 2,3,7,8-TCDD/kg dose level, 70% of the neonates died with overt toxic effects (decreased body weight, liver weight, spleen weight and thymus weight). Bone marrow hypocellularity and depressed macrophages-granulocyte progenitor cells and pleuripotent stem cells were associated with 2,3,7,8-TCDD exposure at the 5.0 and 15.0 μg/kg dose levels. Hematological changes, such as decreased RBC count, hematocrit and hemoglobin, and lymphocyte count showed a dose-related response. Host susceptibility to L. monocytogenes and PYB6-tumor cells was tested in the 2,3,7,8-TCDD-exposed neonates. Death occurred in 73 and 40% of the L. monocytogenes inoculated ($1.2\times10^6$ viable organisms) mice in the 5.0 and 1.0 μg/kg dose groups, respectively, compared with 28% of controls. Tumor development occurred in 44, 60 and 22% of the neonates inoculated with $5\times10^4$ tumor cells from the 5.0 μg 2,3,7,8-TCDD/kg, 1.0 μg 2,3,7,8-TCDD/kg and control groups, respectively.

Hinsdill et al. (1980) reported that 2,3,7,8-TCDD administered in the diet of Swiss-Webster mice at 100 ppb for 5 weeks caused a marked suppression of total serum protein, gamma globulin and albumin, but an increase in β-globulins. At 10 ppb in the diet, 2,3,7,8-TCDD caused decreased immune response to tetanus toxoid, sheep RBC, S. typhimurium and L. monocytogenes, and lowered contact sensitivity to DNFB. This study also sugggested that although young animals are more susceptible to 2,3,7,8-TCDD, older animals are still immunosuppressed and exposure in utero and neonatally is not more crucial than in other periods. Vos and Moore (1974) had previously reported that 1-month-old mice were more sensitive to 2,3,7,8-TCDD than were 4-month-old mice (C57Bl/6). Decreased body weight and thymus weight and spleen cell response to PHA were evident at lower doses in 1-month-old mice than in 4-month-old mice.

The effect of single i.p. doses of 2,3,7,8-TCDD (1, 2, 6 and 30 μg/kg) on peritoneal macrophage and splenic natural killer cell function in mice (C57Bl/6J) was studied by Mantovani et al. (1980) and Vecchi et al. (1980).

2,3,7,8-TCDD treatment at all dose levels did not decrease the cytostatic and cytocidal activity of macrophages or natural killer cells on a per cell basis. The total number of macrophages and splenic natural killer cells recovered from 2,3,7,8-TCDD-treated animals, however, was reduced when compared with untreated controls. Marked hypocellularity noted in the bone marrow of 2,3,7,8-TCDD-treated mice may account for the decrease in peripheral cell counts (McConnell et al., 1978b). The lack of macrophages and natural killer cells was suggested as being instrumental in the decreased resistance to infection common to 2,3,7,8-TCDD-exposed animals (Mantovani et al., 1980). Although 2,3,7,8-TCDD was a strong immunosuppressant, animals given a lethal dose of 2,3,7,8-TCDD did not appear to die from infections, nor did a germ-free environment protect them from death (Greig et al., 1973).

The actual mechanism of 2,3,7,8-TCDD immunotoxicity is unknown but several investigators have tested various hypotheses. Vos et al. (1973, 1978a,b) attempted to address the indirect causes for decreased thymic growth and altered T-lymphocyte activity following 2,3,7,8-TCDD treatment. Vos et al. (1973) measured serum cortisol and corticosteron levels in guinea pigs exposed to 2,3,7,8-TCDD to evaluate the possible indirect immunosuppression by these hormones. There was, however, no significant difference in the level of these hormones between treated and control animals. Indirect immunosuppression of this type was unlikely. Later studies (Vos et al., 1978a,b) investigated the role of thymic hormones (thymosin) on the atrophy of the thymus during 2,3,7,8-TCDD treatment. Thymosin administered in conjunction with 2,3,7,8-TCDD did not protect mice from the typical 2,3,7,8-TCDD-induced immunotoxic alterations. Thymus weight was maintained but not increased by thymosin, and thymus-derived cells continued to show decreased responsiveness to mitogens (PHA, Con A). Thus, it is unlikely that 2,3,7,8-TCDD affects the supply or synthesis of thymic hormones which could lead to the observed immunosuppression.

van Logten et al. (1980) investigated the possible influence of the adrenal gland, hypophysis and pituitary, and growth hormone on thymic atrophy and immunosuppression following 2,3,7,8-TCDD exposure in female F-344 rats. Adrenalectomy and exogenous growth hormone had no preventative action on thymic involution. Hypophysectomized rats showed advanced thymic atrophy.

Sharma and Gehring (1979) noted that 2,3,7,8-TCDD caused stimulation of lymphocyte transformation to blast form cells (mitotically active precursors) when no mitogens were present in the culture system. This represents a phenomenon similar to actual antigenic challenge. At low doses (0.01 and 0.1 μg 2,3,7,8-TCDD/kg/week for up to 8 weeks), serum immunoglobulin levels were elevated in male CD-1 mice. Larger doses of 2,3,7,8-TCDD (1.0 and 10 μg/kg/week) resulted in a decrease in the serum immunoglobulin level. It was suggested that 2,3,7,8-TCDD may elicit an antigenic response either by combining with a body protein or by causing cellular or biochemical damage that releases antigenic proteins. Sharma and Gehring (1979) also noted that thymic atrophy was observed after 2 and 4 weeks of treatment but not after 8 weeks. There may be a recovery of thymic tissue, either by immune tolerance or immune unresponsiveness as a sort of adaptation to 2,3,7,8-TCDD-exposure and its possible antigenic complex.

Luster et al. (1979a,b) reported that 2,3,7,8-TCDD affects the immune system directly by altering lymphocyte function. The function of T-helper cells was not altered, since no change in response to bovine gamma globulin (requires T-helper cell cooperation) was noted in Wistar/Fischer and Fischer rats exposed to 2,3,7,8-TCDD. In vitro, 2,3,7,8-TCDD (100 ng/mℓ) suppressed DNA, RNA and protein synthesis in splenic lymphoid cells from B6C3F1 (Luster et al., 1979a). 2,3,7,8-TCDD, however, did not decrease the binding of $^3$H-Con A to lymphocytes, indicating that these receptors are not blocked by 2,3,7,8-TCDD. T-lymphocytes were more susceptible to 2,3,7,8-TCDD, measured by specific mitogen binding assays, than B-lymphocytes. These authors (Luster et al., 1979a) suggested that 2,3,7,8-TCDD may bind directly to the lymphocyte cell membrane and alter its function. Faith and Luster (1979) reported that lymphocytes from the spleen, thymus, bone marrow and lymph nodes of Fischer rats exposed to 2,3,7,8-TCDD showed abnormal homing patterns within the body. 2,3,7,8-TCDD exposure apparently altered the cell surface markers so that spleen lymphocytes were taken up by the thymus of recipient rats. These authors (Faith and Luster, 1979) suggested that 2,3,7,8-TCDD may change cellular metabolism, which alters the cell membrane constituents or may insert directly into the membrane. Kurl et al. (1982) reported that 2,3,7,8-TCDD causes changes in thymic transcription and RNA synthesis that may lead to cell surface changes. Cell surface changes could presumably result in altered antigen recognition and cell-to-cell recognition, causing immunosuppression and thymic atrophy.

Clark et al. (1981) reported that 2,3,7,8-TCDD treatment (0.4, 4.0, 40 μg/kg weekly for 4 weeks by i.p. injection) caused functional impairment of cytotoxic T-cells in C57Bl/6 male mice. The authors felt that this response was particularly sensitive to 2,3,7,8-TCDD treatment and hypothesized that 2,3,7,8-TCDD directly inhibits the function of these cells. Contrary to the hypothesis tested by these authors and that held by Luster et al. (1979a,b), 2,3,7,8-TCDD treatment impaired the generation of cytotoxic T-cells by the spleen (at doses as low as 0.004 μg/kg when detected *in vitro*) but did not appear directly toxic to the cytotoxic T-cells. At present, the mechanism of immunosuppression caused by 2,3,7,8-TCDD is unknown and the theories available are speculative. In a later study, however, Clark et al. (1983) reported that a 10- to 100-fold greater dose of 2,3,7,8-TCDD was required to suppress cytotoxic T-cells in DBA/2 mice as compared with C56B1/6 mice. This indicates that susceptibility to 2,3,7,8-TCDD immunotoxicity segregates with the Ah locus, which is consistent with a receptor mediated mechanism. The receptor mediated mechanism was further supported by the susceptibility of the C57B1/6 x DBA/2J hybrid mouse to 2,3,7,8-TCDD suppression of the cytotoxic T-cells, which is again consistent with the dominant inheritance of Ah (Nagarkatti et al., 1984).

Few reports are available in which the immunological effects of 2,3,7,8-TCDD exposure were studied in humans. Reggiani (1980) reported that the immunocapability of 17 people, ranging in age from 3-60 years, who had been exposed to 2,3,7,8-TCDD, was normal in all cases. In a survey of 41 workers exposed to 2,3,7,8-TCDD, Ward (1982) measured immunoglobin G, A, M, D and E, as well as lymphocytes, T-cells, B-cells, PHA response and blood cell counts. These determinations were made 10 years after workers had developed 2,3,7,8-TCDD-induced chloracne. In this group of workers, there was a significant increase in the proportion of cases with reduced IgD and IgM. It was suggested that the 2,3,7,8-TCDD-exposed group had a reduced immune capability and a deficiency in T-cell and B-cell cooperation. The immunotoxicity of 2,3,7,8-TCDD in humans cannot be properly assessed because of the paucity of data recorded soon after exposure. The most prominent effects in animals (i.e., humoral responses) were not measured in humans.

### 8.1.1.5. ENZYME INDUCTION BY TCDD --

8.1.1.5.1. In Cell Cultures -- Although 2,3,7,8-TCDD has a very low toxicity to cells in culture (Beatty et al., 1975; Bradlaw et al., 1976; Knutson and Poland, 1980; Yang et al., 1983), it is an extremely potent enzyme inducer in these systems (Kouri et al., 1974; Niwa et al., 1975; Bradlaw et al., 1976; Malik and Owens, 1977; Malik et al., 1979; Bradlaw et al., 1980). This enzyme induction is so sensitive that it has been proposed as a bioassay for detecting planar polychlorinated organic compounds (Bradlaw et al., 1975, Bradlaw and Casterline, 1979; Niwa et al., 1975).

Kouri et al. (1974) found that 2,3,7,8-TCDD induced aromatic hydrocarbon hydroxylase (AHH) activity in cultured human lymphocytes to the same extent as 3-MC; however, the concentration of 2,3,7,8-TCDD necessary for maximal enzyme induction was 40-60 times less than that of 3-MC. Niwa et al. (1975) compared AHH induction by 2,3,7,8-TCDD among cell cultures (H-4-II-E, VERO, HTC, LB82, MA, Hepa-1, TRL2, ERL-2, NRKE and Chang). $ED_{50}$ values ranged from 0.12 nM in the Hepa-1 cell line to >100 nM in the VERO and HTC cell lines. 2,3,7,8-TCDD did not induce AHH activity in LB82 cells. The responsiveness of AHH induction to 2,3,7,8-TCDD was 250-900 times greater than to 3-MC. In addition, cell cultures derived from C57Bl/6N mice were 16 times as sensitive to 2,3,7,8-TCDD as cell cultures derived from DBA/2N mice. The responsiveness of cell cultures to enzyme induction by 2,3,7,8-TCDD is thus similar to the effects seen in vivo. The inductive effect of 2,3,7,8-TCDD was blocked by actinomycin D and cycloheximide, implying that induction involved the sythesis of new mRNA and protein. Enzyme induction by 2,3,7,8-TCDD, therefore, involves an initial RNA synthesis and continuous protein synthesis (Malik and Owens, 1977; Malik et al., 1979).

In all of these studies, there was no correlation between cytotoxicity and enzyme induction. This implies that, despite the correlation *in vivo* (Section 8.3.5.), there may be no direct connection between enzyme induction and the toxicity of 2,3,7,8-TCDD.

8.1.1.5.2. In Mice and Rats -- The effects of 2,3,7,8-TCDD on enzyme activity in rats and mice have been investigated extensively. 2,3,7,8-TCDD has been found to alter many enzyme activities in a wide variety of organ systems (*vide infra*). This alteration primarily results in increased enzyme activity, although 2,3,7,8-TCDD has been observed to inhibit some enzymes.

Hook et al. (1975a) reported that 2,3,7,8-TCDD supressed hepatic microsomal N-demethylation in male, but not female, rats; however, cytochrome P-450 and benzpyrene hydroxylase activity were increased. The suppression of N-demethylase activity was undetectable for 73 days following a single oral dose of 25 µg 2,3,7,8-TCDD/kg bw. The suppression of N-demethylase activity was seen only in adult animals. In 10-day-old rats, 2,3,7,8-TCDD had an inductive effect on this activity.

The inductive effects of 2,3,7,8-TCDD have been demonstrated to be organ specific. Aitio and Parkki (1978) investigated the effects of 2,3,7,8-TCDD on the activities of AHH, ethoxycoumarin deethylase, cytochrome C reductase, epoxide hydratase, UDP glucuronosyltransferase, and glutathione S-transferase in the liver, kidney, lung, small intestine and testes of male Wistar rats. Monooxygenase activity was stimulated in the liver, lung and kidney, but not in any other tissue investigated. UDP glucuronosyltransferase activity increased by a factor of 7 in the liver, by a factor of <2 in the kidney, and not at all in any other tissue. Epoxide hydratase and glutathione S-transferase activities were not affected in any of the tissues studied, although stimulation of hepatic glutathione S-transferase has been reported by other investigators (Manis and Apap, 1979). Enzyme induction has also been reported in rat mammary gland (Rikans et al., 1979), mouse testes (Mattison and Thorgeirsson, 1978), and rat prostate gland (Lee and Suzuki, 1980), but the rat adrenal gland is apparently insensitive to inductive effects of 2,3,7,8-TCDD (Guenthner et al., 1979b).

In the liver of rats and mice, 2,3,7,8-TCDD affects a wide range of enzymatic activities, including DT-diaphorase (Beatty and Neal, 1976a,b), bilirubin catabolism (Kapitulnik and Ostrow, 1978), ornithine decarboxylase (Potter et al., 1982), 7-ethoxycoumarin O-demethylase (Greenlee and Poland, 1978), glutathione S-transferase (Baars et al., 1978; Manis and Apap, 1979), aldehyde dehydrogenase (Lindahl et al., 1978; Deitrich et al., 1977), uroporphyrinogen decarboxylase (Jones and Sweeney, 1977), δ-aminolevulinic acid synthetase (Goldstein et al., 1982a; Woods, 1973), UDP-glucuronosyl transferase (Marselos et al., 1978) and a number of microsomal oxidative enzyme systems (vide infra).

2,3,7,8-TCDD is four orders of magnitude more potent than 3-MC as an inducer of hepatic AHH activity; however, the dose-response curve for the two compounds are parallel and both produce the same maximal response (Poland and Glover, 1974). Simultaneous administrations of maximally inducing doses of both compounds produced no greater response than either alone and both produced a cytochrome with a shift in the absorption maximum of the carbon monoxide difference spectrum from 450 to 448 nm. In a number of studies, increased AHH activity and cytochrome P-448 synthesis have been separated (Chhabra et al., 1976); however, other researchers report an apparent connection between cytochrome P-448 and AHH induction (Kitchin and Woods, 1977, 1978a,b). Thus, 2,3,7,8-TCDD not only stimulates AHH activity by inducing cytochrome P-450 formation, but may enhance AHH activity by other mechanisms as well.

8.1.1.5.3. In Rabbit -- The response of the rabbit is quite different from that observed in rats and mice (Hook et al., 1975a). The only changes in hepatic enzyme activities observed were suppression of benzpyrene hydroxylase and benzphetamine N-demethylase. In the same study, biphenyl 4-hydroxylase was induced in the lung and benzpyrene hydroxylase was induced in the kidney. In a similar study, a hepatotoxic dose of 2,3,7,8-TCDD (30 µg/kg) failed to alter prostaglandin synthetase activity in hepatic or renal tissue (Kohli and Goldstein, 1981).

In a series of studies, Johnson and Muller-Eberhard (1977a,b,c,d), Johnson et al. (1979), Norman et al. (1978a,b), Liem et al. (1980) and Dees et al. (1982) isolated a series of cytochromes P-450 from rabbit liver microsomes. These cytochromes were immunologically distinct, functioned in different catalytic pathways, and responded differently to induction by polycyclic aromatic hydrocarbons. 2,3,7,8-TCDD was found to induce two cytochromes, designated as form 4 and form 6. Form 4 is the major cytochrome induced in adult rabbit liver by 2,3,7,8-TCDD; however, form 6 is the major cytochrome induced in newborn rabbit liver (Norman et al., 1978b), adult rabbit lung, and adult rabbit kidney (Liem et al., 1980; Dees et al., 1982).

8.1.1.5.4. Other Species -- The guinea pig, the species most sensitive to the toxic effects of 2,3,7,8-TCDD, is similar to the rabbit in its response to 2,3,7,8-TCDD. Biphenyl 4-hydroxylase was induced in the liver, lung and kidney, biphenyl 2-hydroxylase was suppressed in the liver, and benzpyrene hydroxylase was induced in the kidney (Hook et al., 1975b). Testicular microsomal cytochrome P-450 content was depressed following a single oral dose of 1 µg/kg, reaching 52% of controls by 1 day and remaining at this level for 9 days (Tofilon et al., 1980). Testicular microsomal heme levels and δ-aminolevulinic acid synthetase activity were unaffected by this treatment. In contrast to the rat, 2,3,7,8-TCDD did not induce DT-diaphorase in brain, spleen, kidney, lung, heart or liver of male guinea pigs (Beatty and Neal, 1978).

Aryl hydrocarbon hydroxylase and δ-aminolevulinic acid synthetase in the chick embryo have been reported to be extremely sensitive to the inductive effects of 2,3,7,8-TCDD (Poland and Glover, 1973a,b), with maximal induction occurring with 155 pmoles/egg. This induction is relatively long lasting, with 70% of the maximum induced activity present 5 days following a single dose of 2,3,7,8-TCDD. Structure-activity studies demonstrated a perfect correspondence between the toxicity and induction potency of a series of dibenzo-p-dioxin congeners (Poland and Glover, 1973a).

8.1.2. Subchronic. Four laboratory studies described the systemic toxic effects of subchronic exposure to 2,3,7,8-TCDD in rodents. Also, one semi-controlled study evaluated the toxic effects to rabbits after confinement to an area containing soil contaminated with 2,3,7,8-TCDD. No information was found in the literature searched on the effects of subchronic exposure to 1,2,3,7,8-PeCDD, and only one preliminary study was available describing the effects of subchronic exposure to a mixture of two HxCDDs in rats and mice.

Kociba et al. (1976) exposed Sprague-Dawley rats to 2,3,7,8-TCDD for 13 weeks. The animals in groups of 12 males and 12 females received the compound suspended in acetone-corn oil (1:9) by gavage 5 days/week at doses of 0.0, 0.001, 0.01, 0.1 or 1.0 μg/kg bw. At the end of the treatment period 5 rats of each sex were killed for histopathologic examination, and the remaining animals were continued for postexposure observation. This report on gross, hematologic, clinical chemistry and histopathologic (on animals terminated at the interim kill or killed when moribund) observations was prepared on data available 13 weeks after termination of treatment. Signs of toxicity were observed only at the two higher dose levels, and female rats appeared more sensitive to the toxic effects of 2,3,7,8-TCDD. During the study there were five treatment-related deaths in the high-dose group females, with three occurring during treatment and two in the post-treatment period. In male animals only two deaths occurred in the post-treatment period in the high-dose group. Both the male and female rats of the 0.1 and 1.0 μg/kg groups had depressed body weight; however, greater relative depression of body weight was observed in the high-dose females. Other changes such as increases in bilirubin concentrations, urinary coproporphyrin excretion, and changes in relative thymus or liver weight to body weight ratio occurred in the two high-dose female groups, but only in the 1.0 μg/kg male group. Although male rats had significantly decreased hematologic values (packed cell volume, RBC count and hemoglobin) in the two high-dose groups, and these values were normal in all female rats, the authors pointed out that these results may have been an artifact resulting from dehydration-induced hemoconcentration in the female rats. No specific data were provided, however, to support this last conclusion.

After necropsy, gross examination revealed subcutaneous edema, a decrease in the size of testes and uteri, and a decrease in the number of corpora lutea. Histologic examination revealed involution of the thymus, decreased number of thymocytes, and focal necrosis and pigment accumulation in the liver. These observations were made only in the animals of the high-dose group, with the exception of a slight decrease in the number of thymocytes and mild microscopic distortion of the architecture of the liver in the group fed 0.1 μg/kg. Although histologic evidence from animals killed during the interim sacrifice was consistent with the liver and thymus being the primary target organs, in an animal that died during the study there were signs of aortic thrombosis and adrenal hemorrhage, and in a second animal there was severe anemia, suggesting possible involvement of the hematopoietic system near the time of death.

Liver toxicity was the only effect of treatment observed during histologic examination of rats (Osborne-Mendel) and mice (B6C3F1) administered 2,3,7,8-TCDD for 13 weeks in a preliminary subchronic toxicity study designed to define an acceptable dose for a chronic toxicity study (NTP 1980a). The animals in groups of 10 males and 10 females were administered the compound in corn oil-acetone (9:1) twice a week at doses for rats of 0.0, 0.5, 1, 2, 4 and 8 μg/kg/week, and for mice at doses of 0.0, 1, 2, 5, 10 and 20 μg/kg/week. Deaths occurred at the two high-dose levels in rats, with 4 females in the 8 μg/kg/week and 1 in the 4 μg/kg/week group dying, while only 2 male rats in the 4 μg/kg/week group died. Deaths were accompanied by severe toxic hepatitis. Hepatic lesions were observed in all other rats examined in groups administered 1-8 μg/kg/week; however, not all animals in each group were submitted to necropsy. Normal liver histology was observed in the 2 male rats examined from the low-dose groups and only threshold toxic effects occurred in the low-dose female rats.

Similar effects of treatment were observed in mice, with a single death occurring in each sex at the high-exposure level, along with reports of hepatic lesions on histologic examination. In contrast to rats, female mice were less sensitive to the hepatotoxic effect of 2,3,7,8-TCDD than were the male mice. Hepatic lesions were observed in all dose groups of male mice, while the 1 and 2 μg/kg/week dose groups of female mice had normal livers. Although the group sizes were small, making conclusions tenuous, it appeared that sex differences in the sensitivity to the toxic effects of 2,3,7,8-TCDD occurred, and that the more sensitive sex may vary with species tested.

In a more extensive subchronic study in rats, King and Roesler (1974) followed the development of toxicity by a series of interim sacrifices during 28 weeks of exposure to 2,3,7,8-TCDD and a 12-week post-treatment recovery period. Groups of 35 male and 35 female Sprague-Dawley rats were intubated twice weekly with 2,3,7,8-TCDD in corn oil-acetone (9:1) at cumulative doses of 0.0, 0.1 and 1 μg/kg/week. No treatment-related deaths occurred; however, 3 animals from each group of each sex were killed after 2, 4, 8 and 16 weeks, and 10 animals of each sex were killed after 28 weeks of treatment. In addition, 3 rats of each sex were killed 4 and 12 weeks after termination of exposure. Animals were monitored for gross changes during the study and were examined for gross and histologic changes at necropsy.

Besides a dose-related decrease in body weight gain in male rats and a decrease in body weight gain in the high-dose female rats, the only effect of exposure to 2,3,7,8-TCDD was histologic changes in the liver. Liver pathology was normal in all treated groups up through the interim kill at 16 weeks. Fatty changes in the liver were considered the most important observation. The fatty changes ranged from single large lipid droplets in a few centrilobular hepatocytes to lipid droplets in all centrilobular hepatocytes with extension into the midzonal hepatocytes. No clear dose-response pattern was observed in this study; however, it did appear that the severity of fatty changes was greater in male rats. During the recovery period, fatty changes progressively decreased in severity but were still present in some treated animals 12 weeks after cessation of exposure. Other histologic changes observed in the liver predominantly in the animals killed at 28 weeks included necrosis, increased nuclear size, subtle distortion of liver architecture, and hyperchromatic nuclei. All of these lesions were considered to be slight or mild, and less toxicologically relevant than the fatty changes. The data suggested that the liver was the most sensitive organ to the toxic effect of 2,3,7,8-TCDD, and although recovery occurred after termination of treatment, the recovery process was slow.

The recovery time was also demonstrated to be long in a subchronic study by Goldstein et al. (1982b) of 2,3,7,8-TCDD induced porphyria. Groups of 8 female Sprague-Dawley rats were given 2,3,7,8-TCDD in corn oil-acetone (7:1) weekly by gavage for 16 weeks at doses of 0.0, 0.01, 0.1 or 10.0 μg/kg/week and killed 1 week after the last treatment. Additional groups of rats received doses of 0.0 or 1.0 μg/kg/week for 16 weeks and were allowed to recover for 6 months. The high-dose level was lethal to all animals within 12 weeks, while the only other gross sign of toxicity was a decrease in body weight gain in the group receiving 1.0 μg/kg/week. After 16 weeks of exposure to 2,3,7,8-TCDD, liver porphyrins were elevated ~1000-fold in 7 of 8 animals receiving 1.0 μg/kg/week, but only 1 of 8 animals in the 0.1 μg/kg/week group had elevated porphyrin levels. No effect was observed in the low-dose animals. After a 6-month recovery period the porphyrin level in animals exposed to 1 μg/kg/week was still 100-fold higher than values in the control group. A similar pattern was observed for urinary excretion of uroporphyrin. The rate-limiting enzyme in heme synthesis, δ-aminolevulinic acid synthetase, was also elevated at both the time of termination of treatment and at the end of the recovery period; however, other enzymes that were increased after 10 weeks of treatment, cytochrome P-450, AHH and glucuronyl transferase, returned to near normal levels by 6 months. It was clear that a 6-month recovery period from subchronic exposure to 2,3,7,8-TCDD at a dose of 1.0 μg/kg/week was not sufficient for complete reversal of 2,3,7,8-TCDD induced porphyria.

In addition to the above laboratory studies, Strik and de Wit (1980) attempted to investigate the toxicologic effect on rabbits of exposure to a natural environment that was contaminated with 2,3,7,8-TCDD. Groups of 20 female rabbits and 1 male rabbit were housed for 5 months in pens, located in five separate areas, on soil that had been contaminated with 2,3,7,8-TCDD. The soil had been cleaned by replacement or cultivation before initiation of the study. The levels of 2,3,7,8-TCDD before cleaning were from 0.8-23.2 μg/m$^3$; however, the levels of contamination after cleaning were not determined. At the end of 5 months liver histology, including the localization of porphyrin, was examined, and the levels of cytochrome P-450 and P-420 were determined along with urinary levels of total porphyrin, creatinine and D-glucaric-acid. All of the parameters examined were considered to be within the normal range. Since exposure data were not available, the negative results of this study cannot be compared with the controlled subchronic laboratory studies already described.

Information on the subchronic toxicity of HxCDD was provided in a preliminary range-finding study for a chronic bioassay conducted by NTP (1980b) on a 1-2 mixture of 1,2,3,6,7,8- and 1,2,3,7,8,9-HxCDD. Osborne-Mendel rats and B6C3F1 mice in groups of 10 males and 10 females were administered the HxCDD mixture in corn oil-acetone (9:1) by gavage twice a week for 13 weeks. The total weekly doses given rats were 0.0, 2.5, 5, 10, 50 and 100 μg/kg; mice received weekly doses of 0.0, 1.25, 2.5, 5, 10 and 50 μg/kg. At week 10 of the study, the body weight in rats was decreased in a dose-related manner to a maximum of ~20% in the high-dose group. In mice, body weight was also decreased 10-20% in the treated animals; however, there appeared to be no correlation with dose. At the end of the study the animals were killed and necropsies were performed on selected animals. In both species liver pathology was observed, with threshold to moderate hepatotoxicity occurring at doses of 5 and 10 μg/kg/week for male and female rats, respectively, and at 10 μg/kg/week for both sexes of mice. At higher exposures, splenic hyperplasia and cortical atrophy of the thymus were also detected in rats. In rats it was unclear whether the low-dose animals were free of any pathologic findings or none were subjected to necropsy. In mice it was stated that no changes were observed in males exposed to 2,3,7,8-TCDD at 1.25 μg/kg/week or in females exposed to 1.25 or 2.5 μg/kg/week. Although the data are limited, it appears that the same target organs are sensitive to the toxic effects of both 2,3,7,8-TCDD and this mixture of HxCDD.

In addition, a second subchronic range finding study conducted by NTP (1980c) evaluated the dermal toxicity of the above mixture of HxCDD. Groups of 10 male and 10 female Swiss-Webster mice were treated by dermal application 3 times/week for 13 weeks. The doses used were from 0.01-50 μg/application with the test compound dissolved in acetone. There was 100% mortality in the 25 and 50 μg/application groups and 80% mortality in the 10 μg/application group. On histologic examination, there were signs of liver damage at the lowest dose tested in both sexes; however, the incidence and degree of damage were not well correlated to the dose applied.

8.1.3. Chronic. The toxic effects, other than neoplasia, of long-term exposure to 2,3,7,8-TCDD have been studied in rats and mice. The primary purpose of many of the studies in rodents was to assess the carcinogenicity of 2,3,7,8-TCDD. The observation of non-neoplastic systemic toxic effects in these studies was often limited, and observations were made near the end of the natural lifespan when conditions associated with aging may have obscured some effects produced by 2,3,7,8-TCDD. Long-duration toxicity assays were also conducted in monkeys. Many of the same organs in monkeys as in rodents were adversely affected by long-term exposure to 2,3,7,8-TCDD; however, the monkeys also developed severe skin and stomach lesions. Table 8-5 summarizes the toxic effects of chronic exposure to 2,3,7,8-TCDD and provides information on the exposure levels that result in the observed effects. There also are data on the chronic toxicity of a mixture of 1,2,3,6,7,8- and 1,2,3,7,8,9-HxCDD. No information was found in the literature search on the effects of chronic exposure to 1,2,3,7,8-PeCDD.

8.1.3.1. STUDIES ON LABORATORY RODENTS -- In an early study, Van Miller et al. (1977a,b) defined the dietary level of 2,3,7,8-TCDD that adversely affected the longevity of rats following chronic exposure. Groups of 10 male Sprague-Dawley rats were maintained for 78 weeks on diets containing 1, 5, 50, 500, 1000, 5000, 50,000, 500,000 or 1,000,000 ppt of 2,3,7,8-TCDD. Survival was monitored during the study or at termination 95 weeks after initiation of treatment. No animals survived until the end of the study at the five highest exposure levels. The respective week after the start of treatment in which the first death occurred in these high-dose groups was 31, 31, 3, 2 and 2 weeks, with all animals in groups >50 ppb dead by week 4. The mortality rate in the 0.0, 1, 5, 50 and 500 ppt groups at 95 weeks was 60, 20, 40, 40 and 50%. Although the small number of animals in each group makes it impossible to precisely define a dose-response relationship, it was apparent that exposure to >1 ppb curtailed survival.

## TABLE 8-5

Effects of Chronic Exposure to 2,3,7,8-TCDD in Laboratory Rodents

| Species/Strain | Sex/No. | Dose | Treatment Schedule | Duration of Study | Parameters Monitored | Effects of Treatment | Reference |
|---|---|---|---|---|---|---|---|
| Rat/ Sprague-Dawley | M/10 | 0.0 ppt | NA | 95 weeks | survival | 40% survived until 95 weeks, the first death occurred at week 68 | Van Miller et al., 1977a,b |
| | M/10 | 1 ppt | continuous in diet for 78 weeks | 95 weeks | survival | 80% survived until 95 weeks, the first death occurred at week 86 | |
| | M/10 | 5 ppt | continuous in diet for 78 weeks | 95 weeks | survival | 60% survived until 95 weeks, the first death occurred at week 33 | |
| | M/10 | 50 ppt | continuous in diet for 78 weeks | 95 weeks | survival | 60% survived until 95 weeks, the first death occurred at week 69 | |
| | M/10 | 500 ppt | continuous in diet for 78 weeks | 95 weeks | survival | 50% survived until 95 weeks, the first death occurred at week 17 | |
| | M/10 | 1000 and 5000 ppt | continuous in diet for 78 weeks | 95 weeks | survival | No animals survived until 95 weeks, the first death occurred at week 31 | |
| | M/10 | 50,000, 500,000 and 1,000,000 ppt | continuous in diet for 78 weeks | 95 weeks | survival | No animals survived until 95 weeks, the first deaths occurred at weeks 2 and 3 | |
| Rats/ Sprague-Dawley | M&F/50&50 | ~2193 ppt (0.1 μg/kg/day) | continuous in diet for 2 years | 2 years | extensive histopathology, hematology, urine analyses, and clinical chemistry | Cumulative mortality, increased (F);<br>bw gain, decreased (M,F);<br>Red blood cell count, decreased (M,F);<br>Packed cell volume, decreased (M,F);<br>Hemoglobin, decreased (M,F);<br>Reticulocytes, increased (M,F);<br>White blood cell count, decreased (F);<br>Serum glutamic pyruvic transaminase, increased (F)<br>G-Glutamyl transferase, increased (F);<br>Alkaline phosphatase, increased (F); | Kociba et al., 1978a, 1979 |

TABLE B-5 (cont.)

| Species/Strain | Sex/No. | Dose | Treatment Schedule | Duration of Study | Parameters Monitored | Effects of Treatment | Reference |
|---|---|---|---|---|---|---|---|
| Rats/ Sprague-Dawley (cont.) | | | | | | Urinary coproporphyrin, increased (F);<br>Urinary uroporphyrin, increased (F);<br>Urinary delta-aminolevulinic acid, increased hepatic degeneration, increased (M,F) | Kociba et al., 1978a, 1979 |
| Rat/ Sprague-Dawley | M&F/50&50 | ~208 ppt (0.01 μg/kg/day) | continuous in diet for 2 years | 2 years | extensive histopathology, hematology, urine analyses and clinical chemistry | Urinary coproporphyrin, increased (F);<br>Urinary uroporphyrin, increased (F);<br>Hepatic degeneration, increased (M,F) | Kociba et al., 1978a, 1979 |
| | M&F/50&50 | ~22 ppt (0.001 μg/kg/day) | continuous in diet for 2 years | 2 years | extensive histopathology, urine analyses and clinical chemistry | No differences in values obtained from control animals | |
| Rat/ Osborne-Mendel | M&F/75&75 | 0.0 μg/kg/week | NA | 106 weeks | extensive histopathology | Toxic hepatitis; 0/74 (M), 0/75 (F) | NTP, 1980a |
| | M&F/50&50 | 0.5 μg/kg/week | administered by gavage biweekly for 104 weeks | 107 weeks | extensive histopathology | Toxic hepatitis; 14/50 (M), 32/50 (F) | |
| | M&F/50&50 | 0.05 μg/kg/week | administered by gavage biweekly for 104 weeks | 107 weeks | extensive histopathology | Toxic hepatitis; 0/50 (M), 1/50 (F) | |
| | M&F/50&50 | 0.01 μg/kg/week | administered by gavage biweekly for 104 weeks | 107 weeks | extensive histopathology | Toxic hepatitis; 1/50 (M), 0/50 (F) | |
| Mice/B6C3F1 | M&F/75&75 | 0.0 μg/kg/week | NA | 105-106 weeks | extensive histopathology | Toxic hepatitis; 1/73 (M), 0/73 (F) | NTP, 1980a |
| | M&F/50&50 | 0.5 μg/kg/week (M)<br>2.0 μg/kg/week (F) | administered by gavage biweekly for 104 weeks | 107 weeks | extensive histopathology | Toxic hepatitis; 44/50 (M), 34/47 (F) | |

TABLE 8-5 (cont.)

| Species/Strain | Sex/No. | Dose | Treatment Schedule | Duration of Study | Parameters Monitored | Effects of Treatment | Reference |
|---|---|---|---|---|---|---|---|
| Mice/B6C3F1 (cont.) | M&F/50&50 | 0.05 μg/kg/week (M)<br>0.2 μg/kg/week (F) | administered by gavage biweekly for 104 weeks | 107 weeks | extensive histopathology | Toxic hepatitis; 3/49 (M), 2/48 (F) | NTP, 1980a |
|  | M&F/50&50 | 0.01 μg/kg/week (M)<br>0.04 μg/kg/week (F) | administered by gavage biweekly for 104 weeks | 107 weeks | extensive histopathology | Toxic hepatitis; 5/44 (M), 1/50 (F) |  |
| Mice/Swiss | M/38 | 0.0 μg/kg/week | NA | 588 days | histology on all organs | Dermatitis and amyloidosis; 0/38 | Toth et al., 1978, 1979 |
|  | M/44 | 0.007 μg/kg/week | administered by gavage weekly for 1 year | 649 days | histology on all organs | Dermatitis and amyloidosis; 5/44 |  |
|  | M/44 | 0.7 μg/kg/week | administered by gavage weekly for 1 year | 633 days | histology on all organs | Dermatitis and amyloidosis; 10/44 |  |
|  | M/43 | 7.0 μg/kg/week | administered by gavage weekly for 1 year | 424 days | histology on all organs | Early mortality, dermatitis and amyloidosis; 17/43 |  |

NA = Not applicable

Increased mortality was also observed in female Sprague-Dawley rats maintained for 2 years on a diet that provided a 2,3,7,8-TCDD dose of 0.1 µg/kg/day, while no increased mortality was observed in male rats at this dose or in animals receiving doses of 0.01 or 0.001 µg/kg/day (Kociba et al., 1978a, 1979). The average dietary levels of 2,3,7,8-TCDD associated with these doses were 2193, 208 and 22 ppt. Interim hematologic, clinical chemistry and urine analyses revealed treatment-related changes in a number of parameters in the high-dose group, along with some of the same changes occurring in the mid-dose group, albeit to a lesser degree (see Table 8-6). At termination of the study, gross and histologic examination indicated that the liver was the most severely affected organ, with degenerative, necrotic and inflammatory changes observed. Increases in urinary excretion rates of coproporphyrin and uroporphyrin in the high and middle dose females were consistent with the observed liver damage. Again, primary liver injury was dose-related with the lowest dose representing a NOEL. Although the group sizes (50 males and 50 females in the treated groups, and 85 males and 86 females in the control groups) were reported, the description of the experimental results did not enumerate the number of animals affected.

When 2,3,7,8-TCDD was administered by gavage in corn oil-acetone (9:1) at dose levels of 0.0, 0.5, 0.05 or 0.01 µg/kg/week, "toxic hepatitis" was observed respectively in male Osborne-Mendel rats at incidences of 0/74, 14/50, 0/50 and 1/50, and in female rats at incidences of 0/75, 32/49, 1/50 and 0/50 (NTP, 1980a). Toxic hepatitis was defined as "lipidosis (lipoidosis) and hydropic degeneration of the cytoplasm of the hepatocytes" in the central, midzonal and, at times, peripheral portions of the liver. No other non-neoplastic lesions were observed even though extensive histologic examinations were performed. The two preceding studies support a NOEL for rats of ~0.001 µg/kg/day, with a LOAEL of 0.05 µg/kg/day, and a FEL for liver injury and possibly decreased survival of 0.5 µg/kg/day.

Non-neoplastic effects of chronic exposure to 2,3,7,8-TCDD in mice have been briefly decribed in studies investigating the carcinogenic potential of 2,3,7,8-TCDD. In an NTP (1980a) bioassay, extensive histologic examinations were performed on B6C3F1 mice treated biweekly with 2,3,7,8-TCDD by gavage in corn oil-acetone (9:1) for 104 weeks followed by an additional 3-week observation period. The doses for male animals were 0.0, 0.01, 0.05 and 0.5 µg/kg/week, and for female animals, the doses were 0.0, 0.04, 0.2 and 2.0 µg/kg/week. The only non-neoplastic lesion was toxic hepatitis, which occurred in males at incidence of 1/73, 5/49, 3/49 and 44/50, and in females at incidences of 0/73, 1/50, 2/48 and 34/47, respectively, in the control, low-, medium- and high-dose groups. In a second study, weekly intubation of 2,3,7,8-TCDD at doses of 0.0, 0.007, 0.7 or 7.0 µg/kg/week for 1 year resulted in amyloidosis of the kidney, spleen and liver, and dermatitis at the time of death in male Swiss mice (Toth et al., 1978, 1979). The incidence of these lesions in the control, low-, medium- and high-dose groups, respectively, was 0/38, 5/44, 10/44 and 17/43. In the high-dose group, the amyloidosis was extensive and considered to be the cause of early mortality. The amyloidosis may have resulted from the chronic dermal inflammation produced by the treatment. From the limited data presented in these studies, it appears that mice and rats were approximately equally sensitive to the toxic effects of 2,3,7,8-TCDD following chronic exposure. Severe toxic effects were observed at doses of 1 µg/kg/day (early mortality) and

0.28-0.07 µg/kg/day (toxic hepatitis), while a LOAEL for dermatitis and amyloidosis of 0.001 µg/kg/day was reported. A NOAEL for mice was not clearly defined by these studies.

The only information available on the effects of chronic exposure to HxCDD was provided by an NTP (1980c) bioassay of a 1:2 mixture of 1,2,3,6,7,8- and 1,2,3,7,8,9-HxCDD. Male and female Sprague-Dawley rats and B6C3F1 mice were exposed biweekly to this mixture for 104 weeks and followed for an additional 3-4 weeks before the terminal kill. Both male and female rats and male mice received doses of 0.0, 1.25, 2.5 and 5.0 µg/kg/week; female mice received doses of 2.5, 5.0 and 10 µg/kg/week. The treated male and female rats had a dose-related decrease in body weight gain during the latter portion of the study, and the high dose females had reduced survival. No gross signs of toxicity were observed in mice of either sex. Although extensive histologic examinations were performed, the only treatment-related effect was toxic hepatitis, which was defined as "degenerative hepatocytic changes and/or necrosis associated with mild fibrosis and infiltration." The incidence of this lesion in control-, low-, medium- and high-dose groups, respectively, was as follows: male rats - 0/75, 28/48, 35/50 and 34/48; female rats - 0/73, 33/50, 37/50 and 44/50; male mice - 0/75, 28/50, 35/50 and 34/49; and female mice - 0/75, 33/50, 37/50 and 44/50. The severity of the toxic hepatitis was dose-related; however, it is unclear how severely the liver was damaged at any of the doses. In rats and mice, all doses of this mixture of 1,2,3,6,7,8- and 1,2,3,7,8,9-HxCDD represented FELs for liver toxicity.

8.1.3.2. STUDIES IN NONHUMAN PRIMATES -- Initial studies indicating the effect of chronic exposure to PCDDs including 2,3,7,8-TCDD in nonhuman primates was conducted using "toxic fat," a contaminated poultry feed additive, which resulted in the death of a large number of chickens (Allen and Carstens, 1967). Groups of 4-5 monkeys, Macaca mulatta, were fed diets containing 0.0, 0.125, 0.25, 0.5, 1.0, 5.0 and 10% toxic fat until death. There was a dose-associated shortening of survival time: monkeys in the high-dose group survived only for an average of 91 days and animals in the low-dose group survived an average of 445 days (data for control animals were not provided). During the course of treatment the animals were monitored for hematologic and gross clinical changes as well as histologic changes in the liver evaluated through needle biopsy samples. At death, major organs were preserved for histologic evaluation. Since both clinical and histologic changes, especially near the time of death, appeared similar regardless of dose, the data and observations were combined for all dose groups.

During the course of the study, the monkeys consumed less food as compared with controls, and progressively lost weight. Gross clinical signs of intoxication during the last 60 to 30 days of life included generalized edema and alopecia. At necropsy, the heart was observed to be hypertrophic and 8 of the 27 animals treated with the "toxic fat" had small gastric ulcers. At the light microscopic level, the liver had developed moderately distorted architecture with vacuolated cells containing neutral fat. The sternal bone marrow was nearly devoid of blood-forming elements, which was consistent with the observed decrease in packed blood cell volume and RBC counts. Also, electron micrographs revealed derangement of the rough endoplasmic reticulum and a loss of ribosomes, which the authors suggested may have resulted in the observed decrease in serum proteins. Skeletal muscle, lungs, GI tract, skin and heart had signs of edema as observed under the light microscope; the electron micrographs of the heart revealed vascular degeneration which, if present in the other tissues, would have accounted for the generalized edema. It was apparent that the active component of "toxic fat" affected many essential biologic processes in the monkey. Chemical analysis of the "toxic fat" has since shown that the fat contained PCDDs of which TCDDs represented 64% by mass (Norback and Allen, 1973).

Allen et al. (1977) also assessed the toxicity of 2,3,7,8-TCDD itself incorporated into the diets of female rhesus monkeys. The animals were maintained for 9 months on diets containing 500 ppt of 2,3,7,8-TCDD, and the animals that survived treatment were observed for an additional 4 months. During the course of the study, the monkeys were observed for clinical signs of toxicity, monitored for hematologic changes and, following death or the termination of the study, were subjected to complete autopsies. Since no control animals were included in this study, the data were compared with pre-exposure values where possible.

As observed in monkeys fed "toxic fat," the monkeys fed 2,3,7,8-TCDD lost hair and developed swollen eyelids and periorbital edema after 3 months of treatment. Blood parameters including hemoglobin levels and hematocrit decreased; however, blood proteins (total serum protein and albumin/globulin ratio) were not altered except in terminal animals. In the three animals that survived the 9-month exposure period, the toxic symptoms continued to develop during the 4 months of observation. The hematologic changes observed during the treatment period were consistent with the microscopic findings at autopsy of bone marrow degeneration. It was suggested that decreased platelet levels resulted in poor clotting and the widespread hemorrhage observed in many organs, which was particularly severe in the stomach. Also, the decreased RBC count and resultant loss of oxygen-carrying capacity resulted in an increase in cardiac workload and hypertrophy of the heart. Cellular hypertrophy, hyperplasia and metaplasia of the epithelium of the salivary gland, bile duct, lung and stomach were also observed microscopically. Although many effects of treatment were observed, it was concluded that the ultimate cause of death was related to the severe pancytopenia.

The total dose of 2,3,7,8-TCDD used over 9 months in this study by Allen et al. (1977) was estimated to be between 2 and 3 μg/kg/day, which is approximately the same dose that resulted in severe toxic effects following chronic exposure in rats and mice. Schantz et al. (1979) reported in an abstract that similar, though less severe, effects were observed in female monkeys following chronic ingestion of diets containing 50 ppt of 2,3,7,8-TCDD. It was also noted that this exposure resulted in a decreased ability to successfully bear young (see Allen et al., 1977, in Section 9). It is apparent that the data available for nonhuman primates do not permit the determination of a NOAEL.

## 8.2. HUMAN

8.2.1. Acute Exposure. Symptoms of acute exposure to materials that contained 2,3,7,8-TCDD are nausea and vomiting, headache and signs of irritation to the eyes, skin and respiratory tract. Acute exposure to chemicals contaminated with 2,3,7,8-TCDD may also result in drenching and sweating with extensive dehydradtion and weight loss, increase in body temperature, severe respiratory distress, fatty degeneration of liver, cyanosis, elevated blood urea nitrogen level, followed by fast deterioration of general condition and death from acute congestive heart failure (Reggiani, 1982; Hay 1982). Initially a chemical burn-type cutaneous reaction will occur (possibly because of other chemicals), usually followed by chloracne after several days to weeks (Taylor, 1979). Chloracne is the most characteristic and frequently observed dermal lesion produced by 2,3,7,8-TCDD and other chlorinated aromatic hydrocarbons in humans (Crow, 1981; Taylor, 1979). This lesion consists of hyperplasia and hyperkeratosis of the interfollicular epidermis, hyperkeratosis of the hair follicle, especially at the infundibulum, and squamous metaplasia of the sebaceous glands that form keratinaceous comedones and cysts (Kimbrough, 1974). These cutaneous eruptions of comedones, cysts and possibly pustules in severe cases, usually occur on the face and shoulders (Crow, 1978a; Passi et al., 1981). The persistence of chloracne varies greatly, with severe cases lasting for up to 15 years, while mild cases may resolve in a matter of months. Similar epidermal changes have been produced by 2,3,7,8- TCDD in rhesus monkeys (McConnell et al., 1978a; Allen et al., 1977), the ear of the rabbit (Poiger and Schlatter, 1980), and hairless mice (Knutson and Poland, 1982). These changes have not generally been observed in other laboratory animals, such as guinea pigs, hamsters, rats and mice.

Chronic exposure to 2,3,7,8-TCDD has probably occurred most in chemical industry workers exposed to low levels of this contaminant during the manufacture of 2,4,5-T on a daily basis. Chloracne is generally the first symptom noted in chronic exposure. Systemic symptoms, including altered function of the neuromuscular system, liver, kidneys, and pancreas, altered blood chemistry (serum bilirubin, GOT, GPT, lipid and cholesterol levels), porphyria cutanea tarda, hyperpigmentation and hyperkeratosis, have also been reported in individuals that have had chronic 2,3,7,8-TCDD exposure (Crow, 1978b, 1981). A combination of acute, high-level exposure to 2,3,7,8-TCDD followed by chronic exposure for many years (or a lifetime) has been noted for residents of areas where PCDDs have been accidentally released into the environment (Taylor, 1979). Residents of Seveso, Italy, for example, where an explosion of a reactor vessel used to manufacture 2,4,5-T released PCDDs and other chemicals into the atmosphere, were exposed acutely for a few days and are now exposed daily to diminishing levels of PCDDs in the soil.

The first cases of chloracne associated with exposure to PCDDs occurred after a 1949 explosion in a chemical factory producing 2,4,5-T in Nitro, WV (Holmstedt, 1980). A total of 228 workers were exposed. Symptoms included nausea, headaches, fatigue, muscular aches and pains, and chloracne (Zack and Suskind, 1980). Chemical tests revealed elevated lipid levels and prolonged prothrombin time. Chronic symptoms, lasting up to 2 years, were severe aches and pains, fatigue, peripheral neuropathy and some residual chloracne. Four additional industrial explosions were reviewed by Holmstedt (1980). In 1953, 75 workers were exposed during an accident at a factory (BASF) in Ludwigshafen, Germany. Most of the workers developed chloracne, while 21 workers developed nervous system and internal organ damage in addition to severe chloracne. In 1963, an explosion at a 2,4,5-T producing factory in Amsterdam resulted in the exposure of 106 men to chlorinated dioxin by-products. Chloracne was the most common symptom, occurring 4-6 weeks after exposure. As a result of a similar exothermic explosion at the Coalite and Chemicals plant (England) in 1968, which manufactured 2,4,5-trichlorophenol, at least 90 workers were exposed to dioxins. Clinical examinations, including liver function tests, full blood counts and urinalysis, were conducted on 14 employees who were in the building at the time of the explosion (May, 1973; Hay, 1982). Eleven of these 14 men showed abnormal liver function (zinc turbidity, thymol turbidity and serum transaminase) and altered hematological parameters or glucosuria. Later, after normal plant operations were resumed, additional workers apparently were exposed to 2,3,7,8-TCDD by contact and developed chloracne. Seventy-nine cases of chloracne developed by the end of 1968. The condition appeared on the face in all cases; however, other parts of the body were affected in more severe cases (May, 1973).

The most recent and extensively studied chemical plant explosion occurred on July 10, 1976, at the ICMESA (Industrie Chimiche-Meda-Societa Azionaria) plant at Seveso, Italy. This accident, caused by the release of the reactor contents into the atmosphere, exposed workers and residents (≥8655 people) of the area to 2,3,7,8-TCDD 2,4,5-trichlorophenol (Garattini, 1982; Pocchiari et al., 1983). A total of 447 patients developed chloracne and some complained of nausea, vomiting, headache, diarrhea, hyperhidrosis and irritation of the eyes (Taylor, 1979). Serious cases of chloracne and dermal blistering occurred in children and appeared within several weeks of their exposure (Gianotti, 1977; Crow, 1981; Taylor, 1979). Pocchiari et al. (1979) cited unpublished data reported to the Lombardy Regional Authority (Boeri, 1978; Chiappino et al., 1978; Sirchia, 1978) on the health effects of 2,3,7,8-TCDD to children and adults at Seveso. Reduced peripheral nerve conduction velocities were noted in adults and children, with abnormalities being more frequent in people residing nearer the chemical plant. The immunology of a group (n=45) of exposed children was compared with a similar unexposed group. No significant differences were noted; however, total serum complement activity, lymphocyte blastogenic response and peripheral blood lymphocytes were elevated to some degree in the exposed children (Tognoni and Bonaccorsi, 1982). Exposure to 2,3,7,8-TCDD has been associated with increased serum glutamate-oxalacetate transaminase (GOT), serum GPT and gamma-glutamyl transferase (g-GT) levels in exposed children

(Pocchiari et al., 1979). Compared with normal values for "healthy" individuals, lympho- cyte aberrations appeared more frequently; however, the findings were not statistically significant.

A comparison between children (under age 15) who developed chloracne and children of the same area who did not develop skin lesions was reported by Caramaschi et al. (1981). A significant increase in the frequency of headaches and eye irritation ($p=0.01$), GI tract symptoms (nausea, vomiting, loss of appetite, abdominal pain or gastritis) ($p=1.6 \times 10^{-4}$), and abnormal g-GT, serum GPT and aminolevulinic acid levels ($p=2.3 \times 10^{-4}$, 0.035 and $1.2 \times 10^{-5}$, respectively) was noted in those children who had chloracne (Caramaschi et al., 1981). Ideo et al. (1982) measured urinary D-glucaric acid levels to assess liver microsomal enzyme activity in 67 children exposed to 2,3,7,8-TCDD at Seveso. A significant ($p<0.05$) increase in the glucaric acid levels, used to indicate increased microsomal enzyme activity, was noted in exposed children 3 years after the accident when compared with unexposed children (n=86).

The decontamination and cleanup of the ICMESA plant at Seveso began in May, 1980, and the possible contamination of clean-up workers was closely monitored and safety measures were implemented (Ghezzi et al., 1982). Laboratory tests on the blood (GOT, GPT, g-GT, alkaline phosphatase, bilirubin, hemoglobin, cell counts, thromboplastic partial time, albumin, gamma globulin, cholesterol and triglycerides) and urine (porphyrin) of the workers were performed and compared with pre-employment values (of the same group of workers) and with a nonexposed group. No significant changes were noted, but exposure to 2,3,7,8-TCDD was believed to be minimal. A recent review of the Seveso incident, including its history and human health effects, is reported by Tognoni and Bonaccorsi (1982).

Three cases of accidental exposure to PCDDs (isomer not specified) while scientists were attempting to prepare a pure standard in the laboratory were reported by Oliver (1975). All three laboratory scientists reported the same general symptoms: chloracne (within several weeks after exposure), GI pains, headaches, fatigability and hypercholesterolemia (occurring 2-3 years after exposure). One case reported loss of mental and muscular coordination and blurred vision. Most symptoms of the patients subsided with time.

Since PCBs and PBBs can cause neurotoxic and behavioral effects (Safe, 1984; Agarwal et al., 1981; Anderson et al., 1978) and their toxic effects may be mediated by the same cytosolic receptor protein as 2,3,7,8-TCDD, it may be important to determine whether 2,3,7,8-TCDD has any neurotoxic activities (Silbergeld, 1984; Safe, 1985).

Additional reports of toxic effects as a result of acute 2,3,7,8-TCDD exposure in humans were noted by Kimbrough et al. (1977). Children were exposed to soil in horse arenas (in Eastern Missouri) sprayed with oil contaminated with 2,4,5-trichlorophenol (5000 ppm in the soil) and 2,3,7,8-TCDD (30 ppm in the soil). A 6-year-old girl developed headaches, diarrhea, epistaxis and hemorrhagic cystitis, and became lethargic. Two 3-year-old boys developed chloracne ~1.5 months after playing in a contaminated horse arena. Three additional individuals who had exposure to the arenas developed less severe symptoms of headache, skin lesions and polyarthralgia. The girl was re-examined 5.3 years following exposure to the soil of the horse arena and showed no residual signs of toxicity (Beale et al., 1977). Additional data on these or other cases from Eastern Missouri were not available.

8.2.2. Chronic Studies. Poland et al. (1971) reported a health survey study of 73 men employed in the manufacture of 2,4,5-T. These workers, however, were also exposed to di- and trichlorophenols, PCDD contaminants and

2,4-D. Thirteen employees developed moderate to severe chloracne; another 35 had minimal "active acne" (cysts, comedones or pustules). Other complaints noted by the workers were eye irritation, hyperpigmentation and hirsutism. Gastrointestinal symptoms (nausea, vomiting, diarrhea, abdominal pain or blood in the feces) were reported by 22 of the 73 workers. Findings as to cardiovascular, hepatic, pulmonary and neurological function were regarded as unremarkable and unrelated to occupational exposures. The authors noted that exposure was to several compounds and to assign a causative agent(s) would be conjecture (Poland et al., 1971).

In a brief report, Walker and Martin (1979) reported on some of the clinical findings of eight men who had contracted chloracne as a result of occupational exposure to 2,3,7,8-TCDD. Five men had elevated g-GT and triglyceride levels. All eight men had decreased levels of high-density lipoprotein (HDL) cholesterol and elevated total/HDL cholesterol ratios consistent with higher than average risk of ischaemic vascular disease. Abnormal lipid levels, reported in 6 men, were attributed to enzyme induction. May (1982), however, observed no differences in triglyceride, cholesterol, alkaline phosphatase D glucaric acid or g-GT levels in 41 workers exposed to 2,3,7,8-TCDD. These determinations were made 10 years after the workers had developed 2,3,7,8-TCDD chloracne.

Bleiberg et al. (1964) found 29 workers in a chemical plant manufacturing 2,4-chlorophenol and 2,4,5-trichlorophenol exhibiting features of chloracne. These patients were tested for the presence of porphyria cutanea tarda (PCT). This investigation revealed evidence of varying degrees of severity of PCT in 11/29 workers, but the authors could not determine any quantitative relationship between the chloracne and PCT. Urinary uroporphyrins were elevated in all 11 cases. A number of workers were noted to have hyperpigmentation, hirsutism, fragility of the skin and vesiculobulbous eruptions on exposed areas of the skin. In this paper the authors suggested PCT is perhaps an acquired disease occurring after various insults to the liver (Bleiberg et al., 1964).

In a survey of 204 employees engaged in the manufacture of 2,4,5-T for 1 month to 10 years, Ott et al. (1980) reported no cases of chloracne, porphyria cutanea tarda or other effects indicative of dioxin exposure. Maximum allowable 2,3,7,8-TCDD levels in the final product were <1 mg/kg in 1966 and <0.1 mg/kg in 1972. Estimates of TWA exposure to 2,4,5-T ranged from 0.2-0.8 mg/m$^3$, so that 2,3,7,8-TCDD levels would be exceedingly low. Cook et al. (1980) reported chloracne, from slight to severe cases, in 49 of 61 employees exposed to 2,3,7,8-TCDD during the manufacture of trichlorophenol. Changes in industrial and personal hygiene techniques decreased potential exposure to 2,3,7,8-TCDD and subsequent chloracne. Additional toxic effects were not reported. The National Institute for Occupational Safety and Health (NIOSH) in a survey of workers at a St. Louis, MO, trucking terminal contaminated with 2,3,7,8-TCDD (subsoil concentration of 2,3,7,8-TCDD was as high as 17 ppb) found one of the long-term former workers had developed porphyria cutanea tarda and angiosarcoma of the right ilium (Hope et al., 1984). Pazderova-Vejlupkova et al. (1981) reported that 80 workers developed chloracne, nausea, fatigue and weakness in the lower extremities while engaged in the production of 2,4,5-sodium trichlorophenoxyacetate and trichlorophenoxyacetate butylester. Prominent clinical symptoms among 55 of the 80 workers included hypercholesterolemia, hyperlipemia and hyperphospholipemia, increased plasma alpha and gamma globulins, and decreased plasma albumin. Porphyria cutanea tarda was observed in 11 of the 55 workers tested. In some cases illness subsided, while other cases became more severe during a 3-4 year follow-up period. Long-term pathological symptoms (remaining evident 5 years after exposure) include deviations in lipid metabolism, abnormal glucose tolerance and high urinary excretion of uroporphyrins (Pazderova-Vejlupkova et al., 1981). Polyneuropathy, usually of the lower extremities, occurred during the period of illness and remained evident after 4 years. Singer et al. (1982) also indicated a decrease in nerve conduction velocities of sural nerves in workers exposed to phenoxy acid herbicides (average exposure, 7 years) when compared with a similar group of nonexposed workers (40.3 m/sec in exposed vs. 42.8 m/sec in nonexposed, p=0.02). Although the causative agent is not known, PCDD contaminants are suggested.

The toxic effects attributed to 2,3,7,8-TCDD exposure were studied over a 10-month period in a group of 78 Vietnam veterans who claimed to have been exposed to Agent Orange (Bogen, 1979). Symptoms reported by the veterans included gastrointestinal complaints (anorexia, nausea, diarrhea, constipation, abdominal pain), joint pain and stiffness, and neurological complaints (numbness, dizziness, headaches, depression and bouts of violent rage). These patients had previously been chronically ill and had frequent infections and allergies (Bogen, 1979). This study was apparently based on personal evaluations of health in a survey-type format. No control group was used for comparison and no clinical or medical evaluations of health were made. Most of these complaints are nonspecific, judgmental and occur commonly in the general public.

In an effort to evaluate the toxic effects attributed to 2,3,7,8-TCDD as a contaminant of Agent Orange, Stevens (1981) estimated a minimum toxic dose of 2,3,7,8-TCDD and determined the amount of this contaminant to which veterans may have been exposed during Agent Orange spraying. Based on studies in which rhesus monkeys were fed small amounts of dietary 2,3,7,8-TCDD and analogy with human data on the minimum toxic dose of 2,3,7,8-tetrachlorodibenzo-p-furan (TCDF), the cumulative minimum toxic dose of 2,3,7,8-TCDD in man was estimated to be 0.1 μg/kg (Stevens, 1981). Based on application rates (4.1 g Agent Orange/$m^2$) and 2,3,7,8-TCDD concentration in the herbicide (2 ppm), the average concentration of 2,3,7,8-TCDD on sprayed surfaces of Vietnam was estimated to be ~8 μg/$m^2$. Based on accidental exposures to 2,3,7,8-TCDD in humans (industrial accidents, Eastern Missouri cases), Stevens (1981) estimated an average intake transfer factor (ratio of absorbed compound to environmentally available compound) of 1:2050 for 2,3,7,8-TCDD. Assuming this absorption-to-exposure ratio and even assuming that a soldier was directly sprayed (exposed to 8 μg/$m^2$) for each day of his 1-year service in Vietnam, his cumulative intake would be only 1.4 μg or 0.02 μg/kg of 2,3,7,8-TCDD (Stevens, 1981). Based on these calculations and assumptions, Stevens (1981) reported that 5 years of direct daily contact with Agent Orange would be necessary to reach a toxic level of 2,3,7,8-TCDD and felt that claims of illness caused by 2,3,7,8-TCDD in Agent Orange were without merit. Exception is made, however, for certain workers (forest industries) who may have been exposed to 2,4,5-T and 2,3,7,8-TCDD for many years.

## 8.3. MECHANISM OF TOXICITY

A number of studies have attempted to determine the mechanism of toxicity of 2,3,7,8-TCDD. The ultimate purpose is to provide a better estimate of man's relative sensitivity to 2,3,7,8-TCDD and other compounds having a similar mode of action. Specifically, these studies may be able to explain the reason for the marked interspecies differences in 2,3,7,8-TCDD toxicity and, thus, help determine if humans possess factors that are associated with sensitivity to 2,3,7,8-TCDD toxicity.

8.3.1. **Receptor-Mediated Toxicity.** Pharmacogenetic studies have played an important role in understanding the biologic and toxic effects of drugs and xenobiotics. Nebert and coworkers have shown that carcinogenic polycyclic aromatic hydrocarbons (PAHs) induce the cytochrome P-450-dependent monooxygenase AHH in certain responsive strains of mice (e.g., C57Bl/6J, BALBc, C3HF/He), whereas this PAH induction activity is minimal or nonexistent in nonresponsive strains (DBA/2J) (Nebert, 1979, 1982; Nebert and Gielen, 1972; Nebert and Jensen, 1979; Nebert et al., 1972, 1981, 1983). The gene complex responsible for the induction of AHH and several other enzymes has been designated the Ah locus that comprises regulatory, structural and possible temporal genes. Extensive studies on genetically inbred responsive and nonresponsive mice (and their backcrosses) indicate that these differences are related to the Aromatic Hydrocarbons (Ah) regulatory gene (termed "Ah complex" or "AH cluster") and its gene product, the Ah cytosolic receptor protein. This receptor protein interacts with PAH ligands and the resultant PAH:Ah receptor complex translocates into the nucleus and presumably initiates the induction of AHH by a process comparable to that proposed for the steroid hormones.

Since the carcinogenic and toxic effects of PAHs are dependent on their oxidative metabolism to reactive electrophilic forms, it is not surprising that the Ah receptor plays an important role in mediating their toxicity and carcinogenicity (Kouri, 1976; Kouri et al., 1974; Benedict et al., 1973; Shum et al., 1979; Thomas et al., 1973; Legraverend et al., 1980; Duran-Reynolds et al., 1978; Robinson et al., 1975; Mattison and Thorgeirsson, 1979). Responsive mice are more susceptible to the toxic (inflammation, fetotoxicity, primordial oocyte depletion) and carcinogenic effects of PAH at organs/tissues in direct contact with the applied chemical; in contrast, nonresponsive mice are more susceptible to the tumorigenic effects of PAHs at tissue/organ sites remote from the initial site of exposure to the PAHs. These differences in susceptibility are due to several factors including AHH-mediated toxication and detoxication.

2,3,7,8-TCDD can produce dermal lesions including epidermal hyperplasia, hyperkeratosis and squamous metaplasia of the sebaceous glands in hairless mice (HRS/J), homozygous for hr/hr locus, but not in heterozygous (hr/+) or normal haired wild type (+/+) mice. These effects on the skin seem to be mediated through the Ah receptor (Poland, 1984).

8.3.1.1. 2,3,7,8-TCDD: SEGREGATION OF ACTIVITY WITH THE Ah LOCUS -- Genetic studies also support the role of the Ah receptor in mediating the toxic and biologic effects of 2,3,7,8-TCDD. Initial studies by Poland and coworkers (Poland et al., 1974, 1983; Poland and Glover, 1975; Nebert et al., 1975) demonstrated that the microsomal AHH-inducing activity of 2,3,7,8-TCDD and 3-MC in several genetically inbred mice strains were similar. Like MC and related PAHs, 2,3,7,8-TCDD induced AHH in several responsive mouse strains (i.e., C57Bl/6J). In contrast to 3-MC, 2,3,7,8-TCDD induced microsomal AHH in the DBA/2J nonresponsive mice; however, the $ED_{50}$ for this biologic response was significantly higher than values reported for the responsive mice. In genetic crosses between responsive C57Bl/6 and nonresponsive DBA/2 mice it was also shown for both 3-MC and 2,3,7,8-TCDD that the trait of responsiveness is inherited in a simple autosomal dominant mode (Poland and Knutson, 1982). It has been suggested that the observed differences in the activities of 3-MC and 2,3,7,8-TCDD are related to their relative Ah receptor affinities (Poland and Knutson, 1982) and the pharmacokinetic and metabolic factors that would more rapidly diminish the "available" concentrations of 3-MC caused by metabolism and excretion.

Several studies with 2,3,7,8-TCDD in genetically inbred mice support the receptor mediated hypothesis. The induction of UDP-glucuranosyl transferase, DT diaphorase, δ-aminolevulinic acid, glutathione-S-transferase B, T-aldehyde dehydrogenase and choline kinase by 2,3,7,8-TCDD or 3-MC in genetically inbred mice have also been shown to segregate with the Ah locus (Beatty and Neal, 1976b; Owens, 1977; Kirsch et al., 1975; Dietrich et al., 1977; Ishidate et al., 1980; Poland and Glover, 1973a). Toxicology studies with genetically-inbred mice confirm the role of the Ah locus in mediating several toxic effects including porphyria, immunotoxicity a wasting syndrome, thymic atrophy and cleft palate formation (Jones and Sweeney, 1980; Poland and Glover, 1980; Courtney and Moore, 1971; Vecchi et al., 1980, 1983). Poland et al. (1982) also linked the tumor-promoting activity of 2,3,7,8-TCDD in hairless mice to the cytosolic receptor. In vitro studies with XB cells in culture also support the role of receptor in mediating a dose-related cell keratinization by 2,3,7,8-TCDD that resembles some of the characteristics of chloracne (Knutson and Poland, 1980). This cell line is also responsive to AHH induction and contains a cytosolic receptor binding protein. Although the murine Ah receptor has not been characterized, several studies confirm that a protein with high affinity for 3-MC and 2,3,7,8-TCDD is present in low concentrations in the hepatic (~30-50 fmolar) and extrahepatic tissues of responsive C57Bl/6J mice (Greenlee and Poland, 1979; Okey et al., 1979, 1980; Poland et al., 1976; Mason and Okey, 1982; Gasiewicz and Neal, 1982; Okey and Vella, 1982; Okey, 1983; Nebert et al., 1983). In responsive C57Bl/6J mice and Sprague-Dawley rats, but not in nonresponsive DBA/2J mice, the Ah receptor can be induced by pretreatment with phenobarbital, which is the only known agent at present that has been demonstrated to affect tissue concentrations of the receptor (Okey and

Vella, 1984). Although the Ah receptor has not been detected in the cytosol of DBA/2J mice, after the administration of radiolabeled 2,3,7,8-TCDD to these mice, some of the radiolabel is detected in the nuclei of the nonresponsive mice. Moreover, the sedimentation characteristics of the [$^3$H]-2,3,7,8-TCDD:nuclear protein complex in DBA/2J mice are similar to those observed with the bound Ah cytosolic receptor protein in C57Bl/6J mice using a sucrose density gradient centrifugation separation technique (Okey, 1983). The cytosolic Ah receptor protein migrates into the nucleus of the cell only after binding with 2,3,7,8-TCDD (Nebert and Jensen, 1979; Nebert, 1980; Greenlee and Poland, 1979; Okey et al., 1979, 1980; Tukey et al., 1982; Gonzalez et al., 1984), and this parallels the observations noted for the interactions between steroids and their receptor proteins. The 2,3,7,8-TCDD inducer-Ah receptor complex undergoes a temperature-dependent step before gaining high affinity for DNA (Okey et al., 1980; Kimura et al., 1984). The 2,3,7,8-TCDD Ah-receptor complex thus binds to the nucleus and regulates the transcription of cytochrome $P_1$-450, which represents the gene product of Ah-structural loci, in mouse hepatoma cells in culture (Whitlock et al., 1984; Eisen, 1984) and in mice with various Ah genotypes (Eisen, 1984). This results in induction of AHH activity which may remain elevated for a prolonged period. Such prolongation of activity may be because cytochrome $P_1$-450 mRNA remains elevated even after 1 week following single exposure to 2,3,7,8-TCDD (Eisen, 1984).

In elucidating the mechanisms of 2,3,7,8-TCDD induced teratogenic effect in the formation of cleft palate in C57 mouse fetus, the presence of Ah-receptor in the palatal shelves of the embryo seems to be necessary for alteration/inhibition of terminal differentiation of the medial epithelial cells in the palate (Denker and Pratt, 1981; Pratt, 1983; Pratt et al., 1984a,b). Pratt and Willis (1985) have even suggested utilizing growth inhibition of an established line of human embryonic palatal mesenchymal cells for *in vitro* short-term screening for assessment of the teratogenic potential of environmental agents.

The presence of Ah-receptor have been detected in normal lung, liver, kidney, spleen and intestine from human fetus. In addition, normal lung tissue from 10 of the 50 individuals examined were found to have Ah-receptor (Roberts et al., 1985). Ah-receptor has also been observed in cell lines of human squamous cell carcinoma at a concentration of 5-10 fmol/mg (Hudson et al., 1983; Roberts et al., 1985). Whether variation in Ah-receptor content in human is genetically determined and is a critical determinant of individual susceptibility to PCDDs is not known and warrants further investigation.

8.3.1.2. 2,3,7,8-TCDD AND RELATED TOXIC HALOGENATED ARYL HYDROCARBONS: STRUCTURE-ACTIVITY CORRELATIONS -- The evidence for a receptor mediated mechanism of action for 2,3,7,8-TCDD is supported by data reported for the effects of other halogenated aryl hydrocarbons in genetically inbred mice and other diverse animal species. A number of reviews and comparative studies (Allen et al., 1979; Kimbrough, 1974; Kimbrough et al., 1978; McConnell and Moore, 1979; Taylor, 1979) clearly indicate that the toxic halogenated mixtures and individual compounds (including the PCDDs, PCDFs, PCBs and PBBs) elicit similar toxic and biologic responses that include 1) a wasting syndrome which is manifested by a progressive weight loss and decreased food consumption by the treated animals; 2) skin disorders including acneform eruptions or chloracne, alopecia, edema, hyperkeratosis, and hypertrophy of the Meibomian glands; 3) lymphoid involution and atrophy; 4) porphyria (resembling porphyria cutanea tarda); 5) endocrine and reproductive disorders; 6) modulation of chemical carcinogenesis; and 7) the induction of numerous enzymes including the cytochrome P-448 (or P-450c) dependent monooxygenases. It is apparent that the effects of these compounds are not manifested in all the animal species tested. McConnell and Moore (1979) summarized the pathologic findings observed in several animal species after pretreatment with PCDDs, PCDFs, PCBs and PBBs; these data illustrate the different species and organ/tissue susceptibilities to these compounds. It is also evident that for most of these effects, all the toxic halogenated aromatics elicit similar effects in these species that also contain the cytosolic receptor protein (Carlstedt-Duke, 1979; Carlstedt-Duke et al., 1979, 1981; Okey, 1983; Okey and Vella, 1982; Mason and Okey, 1982). These observations support a common mechanism of action for all the toxic halogenated aryl hydrocarbons (Poland and Knutson, 1982; Safe et al., 1982; McConnell and Moore, 1979).

Several reports have demonstrated the effects of structure on the activity of PCDDs. The most active member of this group is substituted in the lateral 2, 3, 7 and 8 positions; activity is decreased with 1) decreasing lateral substituents, and 2) increasing Cl substitution. Moreover, for several PCDDs, there is an excellent correlation between the toxicity of individual PCDD congeners in guinea pigs and mice (McConnell et al., 1978b) and their AHH induction potencies in chick embryos and rat hepatoma H-4-II-E cells in culture and their binding affinities for the C57Bl/6J mouse hepatic cytosolic receptor protein (Poland et al., 1976, 1979; Bradlaw et al., 1980; Bradlaw and Casterline, 1979). Comparable structure-activity correlations have been reported for the PCDFs in which the most active compound, 2,3,7,8-TCDF, is an approximate isostereomer of 2,3,7,8-TCDD (Poland et al., 1979; Poland and Knutson, 1982). Moreover, like the PCDDs, there was an excellent correlation among the toxicity of several individual PCDFs (Yoshihara et al., 1981), their AHH induction potencies in rat H-4-II-E hepatoma cells and binding affinities to male Wistar rat hepatic cytosolic receptor protein (Bandiera et al., 1983).

Correlations between structure-activities of PCDDs and Ah-receptor site binding, AHH induction potencies and systemic toxicity have also been suggested (Safe et al., 1984). 2,3,7,8-TCDD, the isomer substituted with Cl in all four lateral positions is most active for all of the above three parameters. Increased or decreased substitution of 2,3,7,8-substituted PCDDs tend to decrease receptor binding affinity and toxic action.

The most active PCB congeners, 3,4,4',5-tetra-, 3,3',4,4'-tetra-, 3,3',4,4',5-penta- and 3,3',4,4',5,5'-hexachlorobiphenyl, are substituted at both para and at two or more meta positions. The four coplanar PCBs induce rat hepatic microsomal AHH and cytochromes P-450a, P-450c and P-450d and resemble 3-MC and 2,3,7,8-TCDD in their mode of induction of the cytochrome P-450 isozymes (34) (Parkinson et al., 1980a,b, 1983; Safe et al., 1982; Sawyer and Safe, 1982; Poland and Glover, 1980; Goldstein et al., 1977). Like Aroclor 1254, all the monoortho and at least eight diortho-chloro analogs of the coplanar PCBs exhibited a "mixed-type" induction pattern and induced microsomal AHH, DMAP N-demethylase and cytochromes P-450a to P-450e (Parkinson et al., 1983, 1980a,c). Quantitative structure-activity relationships (QSARs) within this series of PCBs were determined by comparing their AHH induction potencies ($EC_{50}$) in rat hepatoma H-4-II-E cells and their binding affinities ($ED_{50}$) for the 2,3,7,8-TCDD rat cytosolic receptor protein (Sawyer and Safe, 1982; Bandiera et al., 1983). The results showed that there was an excellent correlation between AHH induction potencies and receptor binding avidities of these compounds and the order or activity was coplanar PCBs (3,3',4,4'-tetra-, 3,3',4,4',5-penta- and

3,3',4,4',5,5'-hexachlorobiphenyls) > 3,4,4',5-tetrachlorobiphenyl > mono-ortho coplanar PCBs > di*ortho* coplanar PCBs. It was also apparent that the relative toxicities of this group of PCBs paralleled their biological potencies (Biocca et al., 1981; Yoshihara et al., 1979; Marks et al., 1981; McKinney et al., 1976; Yamamoto et al., 1976; Ax and Hansen, 1975; Kuroki and Masuda, 1977).

The coplanar and mono*ortho* coplanar PCBs also exhibit differential effects in the inbred C57Bl/6J and DBA/2J mice. These compounds induce AHH and cause thymic atrophy in the former "responsive" mice whereas at comparable or higher doses none of these effects are observed in the nonresponsive DBA/2J mice (Parkinson et al., 1982). The results obtained for structurally diverse PCDDs, PCBs and PCDFs clearly support the role of the receptor protein in initiating the broad spectrum of biologic and toxic effects elicited by these chemicals. Bandiera et al. (1983) demonstrated that the 2,3,7,8-TCDD receptor protein is not only susceptible to halogen substitution patterns but also the structure of the substituent. The cytosol receptor binding avidities and AHH induction potencies in rat hepatoma H-4-II-E cells for several 4'-X-2,3,4,5-tetrachlorobiphenyls were remarkably dependent on the structure of the X substituent. The binding data for 13 different substituents was subjected to multiparameter regression analysis to correlate binding avidities with the physical and chemical characteristics of the critical lateral X substituents. The equation

$$\log \left( \frac{1}{EC_{50}} \right) = 1.53\sigma + 1.47\,\P + 1.09\,HB + 4.08$$

showed that ligand binding was dependent on substituent electronegativity ($\sigma$), lipophilicity ($\P$) and hydrogen binding (HB) with a correlation coefficient (r) equal to 0.978 for 13 different substituents.

Dependency of ligand-receptor complex and the biological activity of PCDDs on their electronic and geometric structure investigated by an _in vitro_ molecular fragment analysis has also been suggested (Cheney, 1982).

The receptor mediated hypothesis for the mechanism of action of 2,3,7,8-TCDD still requires further confirmation and numerous problems must be clarified. For example:

1. Several cell culture lines that appear to have the Ah receptor are highly resistant to the toxicity of TCDD; the nonresponsive HTC and responsive H-4-II-E cell lines (i.e., for AHH inducibility by TCDD) do not possess cytosolic receptor; however, the nonresponsive HTC cells possess more nuclear receptor binding protein than the responsive H-4-II-E cells (Okey, 1983; Okey et al., 1980).

2. Hepatic cytosolic receptor levels in rats (Wistar and Sprague-Dawley), C57Bl/6J mice, hamsters and guinea pigs are comparable (Gasiewicz et al., 1983b); however, their susceptibility to the biologic and toxic effects of TCDD are highly variable: guinea pigs are highly susceptible to the lethal effects of TCDD ($LD_{50}$ = 1-2 μg/kg) whereas the susceptibility of the other species follows the order rat > C57Bl/6J mice > DBA/2J mice > hamster (Neal et al., 1982).

3. "Responsiveness" of the mouse to 2,3,7,8-TCDD induced toxicity seems to be highly dependent on the genetic conditions, as regards the $Ah^b$ allele gene, of the animal. However, cell lines "nonresponsive" to $P_1$-450 induction by 2,3,7,8-TCDD have also been found to possess Ah-receptor protein (Guenthner and Nebert, 1977).

   Ah receptor protein is also present in human tissue (Roberts et al., 1985). Whether variation in Ah-locus is critical for individual susceptibility to toxicity by PCDDs remains to be demonstrated in human population.

8.3.2. **Metabolism.** The metabolism of 2,3,7,8-TCDD has been examined in the guinea pig, rat, mouse and hamster. Urine and bile from $^{14}C$-TCDD-treated animals were found to be free of unmetabolized 2,3,7,8-TCDD, demonstrating that metabolism was required for elimination through these routes (Olson et al., 1983). The direct intestinal elimination of unchanged 2,3,7,8-TCDD in feces suggests, however, that some routes of excretion may not be dependent on prior metabolism of the toxin (Olson et al., 1983).

Thus, it is not possible to directly correlate the half-life for elimination of 2,3,7,8-TCDD with its *in vivo* rate of metabolism in a given species. The relative persistence of 2,3,7,8-TCDD in a given species may be related to the *in vivo* rate of 2,3,7,8-TCDD metabolism, excretion of the toxin not dependent upon metabolism (direct intestinal elimination, lactation, sebum), and the relative tissue distribution of 2,3,7,8-TCDD, particularly to adipose stores. Qualitative and quantitative differences in the metabolism and disposition of 2,3,7,8-TCDD have been observed between various species, and these may in part be related to the remarkable interspecies differences in sensitivity to 2,3,7,8-TCDD toxicity (Olson et al., 1983).

Poiger et al. (1982a) suggested that 2,3,7,8-TCDD metabolism represents detoxification, since they observed relatively little toxicity in guinea pigs given extracts of dog bile containing 2,3,7,8-TCDD metabolites. However, a recent study proposes that metabolites of 2,3,7,8-TCDD may inhibit uroporphyrinogen decarboxylase activity and lead to 2,3,7,8-TCDD-induced porphyria (De Verneuil et al., 1983). Current data on the structural identification of 2,3,7,8-TCDD metabolites suggest that reactive epoxide intermediates may be formed during metabolism (Poiger et al., 1982b; Sawahata et al., 1982). Poland and Glover (1979) reported that the maximum possible *in vivo* covalent binding of 1,6-$^3$H-2,3,7,8-TCDD derived radioactivity to hepatic DNA was 4 orders of magnitude less than the levels of binding observed with other chemical carcinogens. The study found much higher levels of 2,3,7,8-TCDD derived radioactivity bound to hepatic protein of the rat. No data is available, however, on the degree 2,3,7,8-TCDD derived radioactivity is bound to tissues of various species of laboratory animals, which have demonstrated remarkable variability in sensitivity to 2,3,7,8-TCDD. While biliary excretion products may represent detoxified, polar metabolites of 2,3,7,8-TCDD, it remains to be shown whether unexcreted reactive metabolites initiate some of the toxic responses associated with exposure to this toxin.

8.3.3. Vitamin A Depletion. Many of the toxic effects of 2,3,7,8-TCDD resemble the effects of vitamin A deficiency, such as epithelial lesions, keratosis and immunosuppression. The administration of a single oral dose of 0.1, 1.0 or 10 μg 2,3,7,8-TCDD/kg bw produces a dose-related decrease in the hepatic storage of retinol in Sprague-Dawley rats (Thunberg, 1984; Thunburg et al., 1979, 1980). The authors suggested, but did not demonstrate, that the low storage of retinol in the 2,3,7,8-TCDD-treated animals is the result of an increased turnover of retinol.

Hakansson and Ahlborg (1985) pretreated male Sprague-Dawley rats with 2,3,7,8-TCDD at 10 μg/kg bw 4 days before the oral administration of 1200 IU/kg of retinyl acetate. One hundred ninety-two hours postadministration of retinyl acetate the 2,3,7,8-TCDD-pretreated rats excreted 41% of the retinyl acetate compared to the control excreting only 30%. After 2,3,7,7-TCDD treatment the decrease in vitamin A content was 39-53, 19-67 and 18-44% in the liver, intestine and epididymis, respectively. 2,3,7,8-TCDD treatment also influenced vitamin A content in the thymus, initially increasing by 42% in 6 hours and then decreasing by 40% in 192 hours as compared to the controls. 2,3,7,8-TCDD pretreatment increased the vitamin A content in the kidney 3-30 times that of the control. It is important to note that the kidney becomes the primary vitamin A storage organ in vitamin A deficient animals (Johnson and Baumann, 1947; Moore and Sharman, 1950). In a similar study Thunberg and Hakansson (1983) has also found an increase of vitamin A storage in the kidney after a single oral dose of 2,3,7,8-TCDD in male Sprague-Dawley rats., Results from these observations suggest strongly that pretreatment with a single oral dose of 2,3,7,8-TCDD can affect both storage and excretion of retinyl acetate as well as the vitamin A storage in several tissues.

These results suggest that an induced vitamin A deficiency may be responsible for some, but not all, of the toxic effects produced by 2,3,7,8-TCDD. At the highest dose of 2,3,7,8-TCDD, dietary retinol supplements could not fully compensate for the 2,3,7,8-TCDD-produced decrease in hepatic retinol content.

8.3.4. Lipid Peroxidation. Increased lipid peroxidation has been suggested as a possible mechanism of 2,3,7,8-TCDD-induced toxicity (Sweeney and Jones, 1983). This hypothesis is based on the following limited pieces of evidence. First, iron deficiency inhibits *in vitro* lipid peroxidation (Bus and Gibson, 1979; Sweeney et al., 1979) and reduces the hepatotoxic effects of 2,3,7,8-TCDD (Sweeney et al., 1979). Secondly, lipofuscin pigments, by-products of lipid peroxidation, are increased in the heart muscle of rats treated with 2,3,7,8-TCDD (Albro et al., 1978). Thirdly, Sweeney and Jones (1983) reported that administration of the antioxidant butylated hydroxyanisole (BHA) at a level of 0.75% in the diet provided some protection from 2,3,7,8-TCDD-induced prophyria and neutral lipid accumulation. At this dose level of BHA, 4 of the 6 mice (sex not specified) tested were protected; however, at a lower dose (0.25%), all animals were protected from these toxic effects. No beneficial effects were observed when the antioxidant vitamin E (0.01%) was included in the diet.

Recently, Stohs et al. (1983) obtained direct evidence that 2,3,7,8-TCDD accelerates lipid peroxidation in Sprague-Dawley rats. Groups of 4-8 female rats were treated for 3 days with 2,3,7,8-TCDD at doses of 0, 10, 20 or 40 μg/kg by gavage (in a corn oil vehicle). At days 1, 6 and 11 after the last treatment the animals were sacrificed and lipid peroxidation was determined in isolated liver microsomes by the reaction of formed malondialdehyde with thiobarbituric acid. At all sacrifice periods, increased lipid peroxidation was observed and the increase was dose-related. The maximal increase detected on day 6 after the last treatment was 5- to 6-fold greater than in the controls. In addition, these workers measured lipid peroxidation _in vivo_ by the determination of conjugated dienes in rats receiving 2,3,7,8-TCDD at 40 μg/kg. Using this latter method, similar increases in lipid peroxidation were detected, although the maximal increase of 2.35-fold was observed at day 1 postexposure rather than day 6. The authors suggested that the _in vivo_ formation of reactive free radicals during lipid peroxidation could account for the nonspecific nature of 2,3,7,8-TCDD toxicity.

Since β-carotene can quench singlet oxygen ($^1O_2$) and vitamin E is an antioxident, Hassan et al. (1985) studied the effects of vitamins A and E on 2,3,7,8-TCDD induced lipid peroxidation. Vitamin A was found to inhibit lipid peroxidation, elevated the activity of glutathione peroxidate and prevented a 2,3,7,8-TCDD-induced decrease in GSH content in the liver. Vitamin E markedly inhibited microsomal lipid perioxidation, but did not have any effect on glutathione peroxidase activity or glutathione content.

8.3.5. Endocrine Imbalance. Some of the toxic response to 2,3,7,8-TCDD, including hirsutism and diminishing libido, indicate that 2,3,7,8-TCDD may produce some of its toxicity through endocrine disturbances (Oliver, 1975). Nienstedt et al. (1979) reported that a single oral dose of 20 μg 2,3,7,8-TCDD/kg bw significantly reduced testosterone catabolism. Catabolism of exogenous estrogen in ovariectomized rats is also decreased by 2,3,7,8-TCDD pretreatment (Shiverick and Muther, 1982). In this study, there was a 57% increase in serum estrone concentrations following administration of 10 mg estrone/100 g bw/day for 4 days to either control or 2,3,7,8-TCDD pretreated ovariectomized rats. No differences were observed in the increase in uterine wet weight following estrone administration in control and 2,3,7,8-TCDD pretreated rats. Thus, the uterotrophic response was not altered by any 2,3,7,8-TCDD-mediated change in estrone disposition.

Shiverick and Muther (1983) also measured estradiol metabolism in female Holtzman rats given 2,3,7,8-TCDD at a dose of 1 μg/kg bw on days 4-19 of gestation. At this fetal toxic dose, the catechol estrogen formation ability of isolated liver microsomes from the dams was decreased 50% when measured on day 20 of gestation. These microsome preparations had a 4-fold increase in the 7α-hydroxylation of testosterone, while there was no change in the 16α- or 6β-hydroxylase activity. Although steroid metabolism was altered in microsomes isolated from 2,3,7,8-TCDD-treated pregnant rats, similar exposure of pregnant rats on days 4-15 of gestation resultd in no change in circulating levels of serum 17β-estradiol. The authors suggested that other mechanisms besides liver metabolism of steroids may be involved in the fetotoxic effect of 2,3,7,8-TCDD.

Gustafsson and Ingelman-Sundberg (1979) observed that 2,3,7,8-TCDD produced greater change in steroid metabolism in female Sprague-Dawley rats than in male rats of the same strain, resulting in a liver enzyme pattern displaying less sex differentiation than in uninduced rats. Based on this result, they propose that some of the effects of 2,3,7,8-TCDD resulted from an interaction with the hypothalamo-pituitary axis, rather than from a direct effect on steroid metabolism.

Since glucocorticoid hormones are known to have a catabolic effect on lymphoid tissues, such as the thymus and spleen, and these tissues degenerate after exposure of rats to 2,3,7,8-TCDD, Neal et al. (1979) investigated the ability of 2,3,7,8-TCDD to either stimulate the production or mimic the effects of these hormones. In male Sprague-Dawley rats treated by gavage with 2,3,7,8-TCDD at a dose of 50 μg/kg (the ~$LD_{50}$), there was a slight depression in blood glucocorticoids during post-treatment days 1-4, followed by an ~2.5-fold increase on post-treatment days 7 and 14. While in competitive binding assays between 2,3,7,8-TCDD and a synthetic hormone, dexamethasone, 2,3,7,8-TCDD had no affinity for the hormone receptor. Thus, 2,3,7,8-TCDD may have stimulated glucocorticoid production but was not able to mimic the action of these hormones by binding to the glucocorticoid receptor. It was determined, however, that the increase in glucocorticoids was likely not to participate in the toxicity of 2,3,7,8-TCDD through adrenal hyperfunction, since prior adrenalectomy did not provide any protection from the lethal effects of 2,3,7,8-TCDD in rats.

## 8.4. SUMMARY

8.4.1. Experimental Animal Data. A wide range of lethal doses has been reported for 2,3,7,8-TCDD depending on the species tested. The male guinea pig was the most sensitive, with an $LD_{50}$ value of 0.6 μg/kg; the male hamster was the least sensitive, with an $LD_{50}$ value of 5051 μg/kg (Schwetz et al., 1973; Henck et al., 1981). At least for acute exposure, the toxicity of 2,3,7,8-TCDD appears to depend on the total dose administered over a given time and not on whether exposure occurs through a single treatment or a limited number of multiple treatments. Unlike most lethal exposures to toxicants, death resulting from a lethal exposure to a single dose of 2,3,7,8-TCDD occurs long after treatment (5-45 days, see Table 8-1). The most common symptoms after lethal exposure were weight loss, often characterized as "wasting away," and thymic atrophy. Although liver damage was not observed in the guinea pig, the most sensitive species to 2,3,7,8-TCDD, extensive liver damage was reported in rats and mice (Gupta et al., 1973). In general, no specific cause of death could be identified. In a limited comparison of the $LD_{50}$ for 9 congeners of PCDDs, it appeared that biologic activity required chlorine in the 2,3,7,8-positions (McConnell et al., 1978b), with 2,3,7,8-TCDD being the most potent congener.

The liver has been studied extensively with regard to 2,3,7,8-TCDD acute toxicity in rats and mice. Single high doses, 200 μg/kg, of 2,3,7,8-TCDD produced liver necrosis in rats (Jones and Butler, 1974); however, lower doses of 5 and 25 μg/kg produced fatty changes and proliferation of the ER (Fowler et al., 1973). Along with increases in ER, there was an associated marked increase in MFO activity (see Section 8.1.1.5.). Additional membrane changes included degeneration of the plasma membrane with loss of ATPase activity. In species sensitive to the hepatotoxic effects of 2,3,7,8-TCDD, there was also a decreased ability to excrete some xenobiotics into the bile (Yang and Peterson, 1977; Hwang, 1973). Porphyria was also observed, with the mouse being more sensitive than the rat. In addition to effects on the liver, 2,3,7,8-TCDD also affects intestinal absorption by increasing and decreasing the absorption of specific nutrients. In some species, the cellularity of the blood was decreased.

Effects of 2,3,7,8-TCDD exposure on the immune system have been studied extensively. 2,3,7,8-TCDD is undisputably an acute immunotoxic substance in animal models, causing decreases in thymic and splenic weight and hindering, predominantly, cell-mediated immunity. T-lymphocyte function is primarily affected, although a reduction in the immune response to a thymus-independent antigen (type III pneumococcal polysaccharide) has been reported following 2,3,7,8-TCDD exposure (Vecchi et al., 1980). 2,3,7,8-TCDD presumably affects lymphocytes or thymic cells directly, since several studies have negated indirect routes of immunosuppression (hormonal controls). 2,3,7,8-TCDD at immunotoxic levels that alter all function, however, is not directly cytotoxic to lymphocytes (Kociba and Schwetz, 1982). Its effects may be reversible after long recovery periods (Faith and Luster, 1979).

2,3,7,8-TCDD has been shown to alter serum immunoglobin levels in mice at oral doses as low as 0.01 and 0.1 μg/kg/week when administered for up to 8 weeks (Sharma and Gehring, 1979). Thomas and Hinsdill (1979) reported reduced hypersensitivity to DNFB, decreased immune response to _E_. _coli_ LPS and decreased thymic weight in young mice exposed to 2.5 and 5 ppb 2,3,7,8-TCDD (0.33 and 0.65 μg/kg) through maternal dosing. Thigpen et al. (1975) postulated a NOEL of 0.5 μg 2,3,7,8-TCDD/kg/week for 4 weeks, but more precise tests of immunotoxicity suggest a lower NOEL would be appropriate, especially for neonatal and young animals.

The mechanism of 2,3,7,8-TCDD-induced immunotoxicity is not yet known. 2,3,7,8-TCDD is not likely to decrease immune responsiveness through an endocrine control. 2,3,7,8-TCDD may act as an antigenic agent causing immunosuppression and thymic atrophy (Sharma and Gehring, 1979). It has also been suggested that 2,3,7,8-TCDD attaches to the cell membrane of T-lymphocytes, altering the cell surface, which could interfere with antigen and cell-to-cell recognition (Luster et al., 1979a,b; Faith and Luster, 1979).

In subchronic toxicity studies in rats and mice, the liver appeared to be a target organ. The induction of liver damage after repeated exposure to small doses of 2,3,7,8-TCDD was shown in rats. Histologic changes in the liver of rats killed 2, 4, 8, 16 and 28 weeks after exposure to weekly doses of 1 μg/kg bw revealed no fatty changes until week 28; however, 12 weeks after termination of the 28-week exposure, there was still evidence of fatty changes in the liver (King and Roesler, 1974). A similar long induction period was observed by Goldstein et al. (1982b) for porphyrin accumulation in the liver of rats. Following 16 weeks of exposure to 2,3,7,8-TCDD and a 6-month postexposure period, porphyrin levels were still elevated. The only study in mice (NTP, 1980a) described toxic hepatitis as the only effect of subchronic exposure to low levels of 2,3,7,8-TCDD. In these and other subchronic studies, NOELs of 0.01 µg/kg/day (Kociba et al., 1976), 0.5 µg/kg/week (NTP, 1980a) and 0.01 µg/kg/week (Goldstein et al., 1982b) have been reported for rats. In mice, a NOEL of 2 µg/kg/week was obtained in females, while males exposed to 1 µg/kg/week (the lowest dose tested) developed toxic hepatitis. Similar hepatic lesions were observed after exposure to a mixture of HxCDDs with NOELs of 2.5 and 1.25 µg/kg/week reported for rats and mice, respectively (NTP, 1980b).

In chronic toxicity studies in rats and mice, it was again the liver that appeared to be the most sensitive organ. Changes in the liver of rats included initially fatty infiltration, and at higher doses, necrosis. The studies in rats indicated that 0.001 µg/kg/day was a NOEL, while 0.05 and 0.1 µg/kg/day were the NOAEL and FEL for liver damage (Kociba et al., 1978b, 1979; NTP, 1980a). In mice, a NOEL was not determined, with the lowest doses tested, 0.0015 and 0.006 µg/kg/day, producing liver damage in male and female B6C3F1 mice (NTP, 1980a), while the lowest dose tested in Swiss mice, 0.001 µg/kg/day, produced amyloidosis of the kidney, spleen and liver (Toth et al., 1978, 1979). In nonhuman primates, chronic exposure to 2,3,7,8-TCDD in the diet at 50 or 500 ppt resulted in hair loss, edema and pancytopenia (Allen et al., 1977; Schantz et al., 1979). Data were not available to determine a NOEL for monkeys. Also, in the only study available for 1,2,3,6,7,8- or 1,2,3,7,8,9-HxCDD, the lowest doses tested, 1.25 and 2.5 µg/kg/week for males and females, respectively, produced toxic hepatitis and represented a FEL (NTP, 1980b).

8.4.2. Human Data. There seems to be general agreement that exposure to 2,3,7,8-TCDD, whether acutely or chronically, leads to chloracne, altered liver function, hematological abnormalities, porphyria cutanea tarda, hyperpigmentation and hirsutism. Recently, Suskind and Hertzberg (1984) have demonstrated an association between exposure to 2,4,5-T contaminated with 2,3,7,8-TCDD and the history of GI ulcer. No evidence of increased risk for cardiovascular disease, hepatic disease, renal damage or central or peripheral nervous system problems could be found in a group of workers exposed to 2,4,5-T following a run way reaction (Suskind and Hertzberg, 1984). However, occupational or accidental exposure to 2,3,7,8-TCDD has been shown to produce neurological ailments in addition to the above ailments. The neurological problems include peripheral polyneuropathies, impairment of sensory functions including sight disorders, loss of hearing, taste and sense of smell, central lassitude, weakness, impotence and loss of libido (Reggiani, 1982; Kimbrough et al., 1984). Only one estimate was available, which speculates a cumulative minimum toxic dose of 0.1 µg/kg for man (Stevens, 1981). The available follow-up reports and epidemiological studies, primarily on populations exposed occupationally, accidentally or in Vietnam, indicate that toxic effects noted soon after exposure to 2,3,7,8- TCDD may subside or may persist for many years.

8.4.3. Mechanisms of Toxicity. In the preceding sections, five possible mechanisms by which 2,3,7,8-TCDD may produce its toxic effects were reviewed. The data suggest that metabolism of 2,3,7,8-TCDD is a detoxification process, resulting in the production of metabolites that are less toxic than the parent compound, although intermediate or minor metabolites of 2,3,7,8-TCDD may be involved in toxicity. Vitamin A depletion, increased lipid peroxidation and effects on the hypothalamo-pituitary axis have all been implicated as possible mechanisms for 2,3,7,8-TCDD-induced toxic response. It seems probable that these mechanisms are responsible for some, but not all, of the toxic effects of 2,3,7,8-TCDD.

The major mechanism of 2,3,7,8-TCDD toxicity that has received intense investigation involves effects mediated by specific cytosolic receptors produced by the Ah locus. The toxicity of various dioxins has been correlated with binding to the cytosolic receptor and enzyme induction in a wide range of animal species and under a variety of experimental conditions (vide ante). While these studies have been done in several species, species differences in the toxic response to 2,3,7,8-TCDD do not correlate with species differences in receptor concentration or affinity, or with the degree of enzyme induction. It thus appears that the toxicity of 2,3,7,8-TCDD may be mediated by binding to the cytosolic receptor responsible for enzyme induction; however, this theory does not apply in various species, and cell culture studies indicate that enzyme induction is not necessarily a cytotoxic process.

# 9. TERATOGENICITY AND OTHER REPRODUCTIVE EFFECTS

## 9.1. STUDIES ON EXPERIMENTAL MAMMALS

### 9.1.1. 2,3,7,8-TCDD Administered as a Contaminant of Other Chemicals.

Courtney et al. (1970a,b) were the first to report that 2,4,5-T was capable of causing teratogenic effects in rats and mice. In these studies, rats and two strains of mice were exposed subcutaneously or orally to 2,4,5-T containing 30 ppm 2,3,7,8-TCDD. The mixture was teratogenic and fetotoxic to mice at $\geq$46.4 mg/kg. Rats were more sensitive, exhibiting fetotoxic responses at 10 mg/kg for this 2,4,5-T/2,3,7,8-TCDD mixture. Since this initial report, research has focused on determining the role of 2,3,7,8-TCDD contamination in eliciting the teratogenic response. These studies are summarized in Table 9-1.

Neubert and Dillmann (1972) conducted a detailed study to determine the significance of 2,3,7,8-TCDD contamination. These investigators assayed three 2,4,5-T samples: a highly purified sample containing <0.02 ppm 2,3,7,8-TCDD (referred to as Sample A), a purified sample identical to that used by Roll (1971) that contained 0.05±0.02 ppm 2,3,7,8-TCDD (Sample B), and a commercial sample containing an undetermined quantity of 2,3,7,8-TCDD (Sample C). All three samples induced cleft palates at sufficiently high doses (30-90 mg/kg). In terms of the number of fetuses with cleft palate/the total number of fetuses, the dose/response pattern observed by Neubert and Dillmann (1972) was similar to that observed by Roll (1971) using a similar grade of 2,4,5-T. In addition to the three 2,4,5-T samples, Neubert and Dillmann (1972) also assayed a sample of 2,3,7,8-TCDD alone and in various combinations with the highly purified sample of 2,4,5-T. This approach allows at least partial quantification of the significance of 2,3,7,8-TCDD contamination in 2,4,5-T-induced cleft palates. When the

TABLE 9-1

Studies on the Potential Teratogenic Effects of 2,3,7,8-TCDD Contaminated 2,4,5-T

| Species/Strain | Vehicle | Form of 2,4,5-T | TCDD Level | Daily Dose | Treat-ment Days | Obser-vation Day | Maternal Response | Fetal Response | Reference |
|---|---|---|---|---|---|---|---|---|---|
| Mice/NMRI | Rape-seed oil | acid | <0.02 ppm (Sample A) | 8, 15, 30, 45, 60, 90 and 120 mg/kg | 6-15 | 18 | No toxic effects; decreased maternal weight at doses of 90 mg/kg and greater | Significant increases in the incidence of cleft palates at doses above 30 mg/kg (see text for additional details). Significantly decreased ($p<0.005$) fetal weight at all dose levels. | Neubert and Dillmann, 1972 |
| Mice/NMRI | Rape-seed oil | acid | 0.05±0.02 ppm (Sample B) | 30, 60 and 90 mg/kg | 6-15 | 18 | No toxic effects; decreased maternal weight at 90 mg/kg | Increases in the incidence of cleft palate at 60 and 90 mg/kg; significant ($p<0.005$) at all dose levels | Neubert and Dillmann, 1972 |
| Mice/NMRI | Rape-seed oil | acid | NR (Sample C) | 90 mg/kg | 6-15 | 18 | No toxic effects but decreased maternal weight | Increase in the incidence of cleft palate; significant ($p<0.005$) decrease in fetal weight | Neubert and Dillmann, 1972 |
| Mice/NMRI | Rape-seed oil | butyl ester | NR | 12 and 17 mg/kg | 6-15 | 18 | No toxic effects | Significant decrease in fetal weight but no effect on mortality; increase in the frequency of cleft palate similar to that seen with acid (see text) | Neubert and Dillmann, 1972 |
| Mice/NMRI | NR | acid | 0.05±0.02 ppm | 20, 35, 60, 90 and 130 mg/kg | 6-15 | NR | Toxic effects observed at 90 and 130 mg/kg | Increases in the percentage of resorptions and/or dead fetuses at 90 and 130 mg/kg; increases in the incidence of cleft palate and retardation of skeletal development at 35 mg/kg and above | Roll, 1971 |
| Mice/CD-1 | Corn oil:acetone (9:1) | acid | <0.05 ppm | 115 mg/kg | 10-15 | 18 | No significant effect on weight gain or liver-to-bw ratios | No effect on fetal mortality or fetal weight but an increase in the incidence of cleft palate | Courtney, 1977 |

TABLE 9-1 (cont.)

| Species/Strain | Vehicle | Form of 2,4,5-T | TCDD Level | Daily Dose | Treatment Days | Observation Day | Maternal Response | Fetal Response | Reference |
|---|---|---|---|---|---|---|---|---|---|
| Mice/C57BL/6 | Honey:water (1:1) | acid | 30 ppm | 46.4 and 113 mg/kg | 6-14 | 18 | NR | Significant ($p<0.01$) increases in the incidence of cleft palate in the high dose group and cystic kidney in both dose groups; increased fetal mortality also observed in the high dose group | Courtney et al., 1970a,b |
| Mice/AKR | Honey:water (1:1) | acid | 30 ppm | 113 mg/kg | 6-15 | 19 | Increase in liver-to-bw ratio | Significant ($p<0.05$) increases in the incidence of cleft palate and fetal mortality | Courtney et al., 1970a,b |
| Rats/Sprague-Dawley (groups of 25 rats) | Gavage/hydroxy-propyl-methyl-cellulose | acid | 0.5 ppm | 1, 3, 6, 12 or 24 mg/kg/day | 6-15 | 20 | No effect on bw and no observable signs of toxicity | A slight but statistically significant ($p<0.05$) decrease in implantations and litter size in lowest dose group only; no frank teratogenic effects based on a detailed examination of the control and 24 mg/kg dose group; the only effect noted was an increase in the incidence of 5th partially ossified sternebrae | Emerson et al., 1970, 1971 |
| Rats/Wistar | Gavage/aqueous gelatin or corn oil | acid | <0.5 mg/kg | 25, 50, 100 or 150 mg/kg/day | 6-15 | 22 | Some maternal mortality and decreased bw gain at 150 mg/kg; no signs of toxicity at 100 mg/kg or below | At 100 or 150 mg/kg, decreased fetal weight, increased fetal mortality and an increase in the incidence of skeletal anomalies; no significant effect at the two lower dose levels | Khera and McKinley, 1972; Khera et al., 1971 |
| Rats/Wistar | Gavage/aqueous gelatin or corn oil | butyl ester | <0.5 mg/kg | 50 or 150 mg/kg/day | 6-15 | 22 | NR | No significant effect on fetal mortality, fetal weight or the incidence of anomalies | Khera and McKinley, 1972; Khera et al., 1971 |

TABLE 9-1 (cont.)

| Species/Strain | Vehicle | Form of 2,4,5-T | TCDD Level | Daily Dose | Treatment Days | Observation Day | Maternal Response | Fetal Response | Reference |
|---|---|---|---|---|---|---|---|---|---|
| Rats/Holtzman | Gavage/1:1 solution of honey and water | acid | 30 ppm | 4.6, 10.0 and 46.4 mg/kg/day | 10-15 | 20 | NR | Significant ($p<0.01$) increases in fetal mortality at the 2 higher dose levels; dose-related increases in the percent of abnormal fetuses per litter; a high incidence of cystic kidneys in treated groups | Courtney et al., 1970a,b |
| Rats/CD | Gavage/15% sucrose solution | acid | 0.5 ppm | 10.0, 21.5, 46.4 and 80.0 mg/kg/day | 6-15 | 20 | Reduced maternal weight gain at the 2 higher dose levels ($p<0.05$) and increased liver-to-bw ratio at the highest dose level ($p<0.05$) | Increase in the incidence of kidney anomalies, but no increase in cleft palate | Courtney and Moore, 1971 |
| Rats/strain not specified | Gavage/methocel | acid | 0.5 ppm | 50 mg/kg | 6-15 | NS | No effect on mortality or bw gain | No significant effect on fetal mortality or fetal weight; a significant ($p<0.05$) increase in the incidence of delayed ossification | Sparschu et al., 1971a |
| Rats/strain not specified | Gavage/methocel | acid | 0.5 ppm | 100 mg/kg | 6-10 | NS | Increased mortality and decreased bw gain | Increase in the incidence of delayed ossification and poorly ossified or malaligned sternebrae ($p<0.05$) | Sparschu et al., 1971a |
| Syrian hamsters/ *Mesocricetus euratus* | Gavage/acetone, corn oil, and carboxymethyl cellulose in ratio of 1:5.8:10 | acid | <0.1-4.5ppm | 20, 40, 80 and 100 mg/kg | 6-10 | 14 | NS | Dose-related increases in fetal mortality, gastrointestinal hemorrhages, and fetal abnormalities; see text for discussion of effect TCDD level on development | Collins et al., 1971 |

NS = Not specified; NR = Not reported

litter is used as the basic experimental unit, the incidences of cleft palate (number of litters with cleft palate/total numbers of litters) versus the dose can be plotted on log dose/probit response paper, correcting for background response using Abbott's equation. According to this method, the $ED_{50}$ (by eye-fit) for cleft palate induction are as follows:

2,3,7,8-TCDD: 4.6 μg/kg bw
2,4,5-T (Sample A): 115 mg/kg bw
2,4,5-T (Sample B): 46 mg/kg bw

If the assumption were made that all teratogenic activity in the 2,4,5-T samples were attributable to 2,3,7,8-TCDD contamination, the expected $ED_{50}$ for samples A and B would be 230,000 mg/kg (0.0046 mg/kg x 0.02 $\text{ppm}^{-1}$) and 92,000 mg/kg (0.0046 mg/kg x 0.05 $\text{ppm}^{-1}$), respectively. Since the observed $ED_{50}$ was lower by a factor of over 1000, this suggests that 2,3,7,8-TCDD is not the sole factor in 2,4,5-T-induced cleft palate.

The nature of possible interaction between 2,4,5-T and 2,3,7,8-TCDD is more difficult to define. Based on assays of five mixtures of 2,3,7,8-TCDD and the highly purified 2,4,5-T, Neubert and Dillmann (1972) noted a greater than additive effect on the induction of cleft palates. A similar conclusion can be reached if one assumes that Sample A was a "totally pure" sample of 2,4,5-T. Using the assumptions of simple similar action (Finney, 1971) and treating Sample B as a mixture of 2,3,7,8-TCDD and 2,4,5-T, the expected $ED_{50}$ for Sample B would be 119.8 mg/kg. The observed value of 46 mg/kg again suggests a greater than additive effect. A more detailed statistical analysis of these data, however, would be required to support the assumptions of simple similar action or independent joint action that are implicit in these analyses. Furthermore, the inability to define precisely the levels of 2,3,7,8-TCDD in the 2,4,5-T samples and the possible significance of other contaminants would preclude an unequivocal interpretation of the results of the analysis.

Nevertheless, three of the studies summarized in Table 9-1 (Neubert and Dillmann, 1972; Roll, 1971; Courtney, 1977) have demonstrated the induction of cleft palate in mice by using 2,4,5-T samples containing 2,3,7,8-TCDD levels of 0.05 ± 0.02 ppm or less. Although 2,3,7,8-TCDD contamination is undoubtedly a factor in the teratogenic activity of 2,3,7,8-TCDD contaminated 2,4,5-T, the above analysis suggests that 2,3,7,8-TCDD contamination is not the sole factor, and that some teratogenic activity must be attributed to 2,4,5-T itself or other contaminants in 2,4,5-T.

**9.1.2. 2,3,7,8-TCDD Studies in Mice.** Courtney and Moore (1971) tested a purified sample of 2,3,7,8-TCDD for teratogenic potential. A summary of this study and others assessing the teratogenic potential of purified 2,3,7,8-TCDD are presented in Table 9-2. CD-1, DBA/2J and C57Bl/6J mice were given subcutaneous injections of 2,3,7,8-TCDD at 1 or 3 μg/kg/day on days 6-15 of gestation in the study by Courtney and Moore (1971). This dose regime did not result in maternal toxicity, although an increase in the maternal liver/bw ratio was observed in DBA/2J and C57Bl/6J mice. 2,3,7,8-TCDD had no measurable effect on fetal mortality; however, anatomical abnormalities were observed in all strains and at all dose levels, with C57Bl/6J being the most sensitive strain. The abnormalities observed were cleft palate and unspecified kidney anomalies.

Moore et al. (1973) treated pregnant C57Bl/6 mice with an oral dose of 2,3,7,8-TCDD at 1 or 3 μg/kg/day on days 10-13 of gestation, or 1 μg/kg on day 10 of gestation. At the high dose level, the average incidence of cleft palate was 55.4%. Kidney anomalies (hydronephrosis) were observed on

TABLE 9-2

Studies on the Potential Teratogenic Effect of 2,3,7,8-TCDD

| Species/Strain | Vehicle | Daily Dose | Treatment Days | Observation Day | Maternal Response | Fetal Response | Reference |
|---|---|---|---|---|---|---|---|
| Mouse/C57Bl/6<br>Mouse/AKR | DMSO or honey:water (1:1) | 21.5, 46.4, 113.0 mg/kg | 6-14 or 9-17 | $19^a$ | increased liver/ bw ratio | fetocidal, cleft palate, cystic kidney | Courtney et al., 1970b |
| Mouse/CD-1<br>Mouse/DBA/2J<br>Mouse/C57Bl/6J | DMSO | 0.5, 1, 3 µg/kg | 6-15 | $17^a$ or 18 | increased liver/ bw ratio | cleft palate, kidney anomalies | Courtney and Moore, 1971 |
| Mouse/C57Bl/6 | acetone: corn oil (1:9) | 1, 3 µg/kg | 10-13 or 10 | $18^a$ | none reported | cleft palate, kidney anomalies | Moore et al., 1973 |
| Mouse/CD-1 | DMSO or corn oil | 25, 50, 100, 200, 400 µg/kg | 7-16 | $18^b$ | increased liver/ bw ratio | cleft palate, hydronephrotic kidneys, hydrocephalus, open eyes, edema, petechiae | Courtney, 1976 |
| Mouse/CF-1 | corn oil/ acetone (98:2) | 0.001, 0.01, 0.1, 1.0, 3.0 µg/kg | 6-15 | $18^a$ | none reported | cleft palate, dilated renal pelvis | Smith et al., 1976 |
| Mouse/NMRI | rape-seed oil | 0.3, 3.0, 4.5, 9.0 µg/kg | 6-15 | 18 | no effect observed | fetocidal at the high dose, cleft palate at doses at or above 5 µg/kg | Neubert and Dillmann, 1972 |
| Rat/CD | DMSO | 0, 0.5, 2.0 µg/kg | 6-15, 9 and 10, or 13 and 14 | $20^a$ | none reported | kidney malformations at both dose levels | Courtney and Moore, 1971 |
| Rat/Sprague-Dawley | corn oil/ acetone | 0, 0.03, 0.125, 0.5, 2.0 and 8.0 µg/kg | 6-15 | $20^a$ | vaginal hemorrhage at 2.0 and 8.0 µg/kg | intestinal hemorrhage at 0.125 and 0.5 µg/kg, fetal death at higher doses, subcutaneous edema | Sparschu et al., 1971b |
| Rat/Wistar | corn oil/ anisole | 0.0, 0.125, 0.25, 1, 2, 4, 8, 16 µg/kg | 6-15 | 22 | maternal toxicity observed at or above 1 µg/kg | increased fetal death observed at or above 1 µg/kg, subcutaneous edema and hemorrhages in the 0.25-2 µg/kg groups | Khera and Ruddick, 1973 |

TABLE 9-2 (cont.)

| Species/Strain | Vehicle | Daily Dose | Treatment Days | Observation Day | Maternal Response | Fetal Response | Reference |
|---|---|---|---|---|---|---|---|
| Rat/Sprague-Dawley | corn oil/ acetone (9:1) | 0.1, 0.5, 2.0 μg/kg | 1-3 | 21 | decrease in bw gain in the high dose group | decreased fetal weight in the 0.5 and 2 μg/kg group | Glavini et al., 1982a |
| Rat/Sprague-Dawley | diet | 0.001, 0.01 and 0.1 μg/kg[c] | throughout gestation | post-parturition | low fertility at 0.01 and 0.1 μg/kg decreased bw at 0.01 and 0.1 μg/kg dilated renal pelvis | low survival at 0.01 and 0.1 μg/kg, decreased bw at 0.01 μg/kg, slight dilated renal pelvis at 0.001 μg/kg in the $F_1$ but not succeeding generations[d] | Murray et al., 1979 |
| Rabbit/ New Zealand | corn oil/ acetone (9:1) | 0.0, 0.1, 0.25, 0.5 and 1 μg/kg | 6-15 | 28 | maternal toxicity at doses of 0.25 μg/kg and above | increases in extra ribs and total soft tissue anomalies | Glavini et al., 1982b |

[a] First day of gestation designated day zero

[b] First day of gestation designated day one

[c] The high dose level (0.1 μg/kg/day) was discontinued due to very low fertility in adults

[d] Nisbet and Paxton (1982) re-evaluated the study by Murray et al. (1979) using different statistical methods and considered the effects in the 0.001 μg/kg group to be statistically significant.

an average of 95.1% of the fetuses/litter, with 83.1% having bilateral kidney anomalies. When the dose was decreased to 1 µg/kg/day, the average incidence of cleft palate dropped to 1.9%; however, the incidence of kidney anomalies remained relatively high, with an average incidence of 58.9%. On the average, bilateral kidney anomalies occurred in 36.3% of the fetuses/litter. A single dose of 1 µg/kg on day 10 of gestation produced kidney anomalies in 34.3% of the fetuses; however, no cleft palates were observed. When C57Bl/6 mice were treated with 1 µg/kg on day 10 of gestation and were then allowed to litter, the detection of kidney lesions on postnatal day 14 was found to depend largely on whether the pups nursed on a 2,3,7,8-TCDD-treated mother. When pups from a 2,3,7,8-TCDD-treated mother nursed on control mice, kidney anomalies were found in only 1/14 litters. In contrast, when pups from control mothers nursed on 2,3,7,8-TCDD-treated mice, kidney anomalies were observed in 4/14 litters. In the pups exposed to 2,3,7,8-TCDD both _in utero_ and during the postnatal period, kidney anomalies were observed in 5/7 litters. Kidney anomalies observed following _in utero_ exposure or exposure through the milk were similar, and these kidney anomalies may not be considered a purely teratogenic response.

Neubert et al. (1973) reviewed what was known of the embryotoxic effects of 2,3,7,8-TCDD in mammalian species. Also reported were their own studies and previous work (Neubert and Dillmann, 1972) using NMRI mice, in which cleft palate was observed to be a common abnormality; however, no kidney anomalies were reported. Neubert and Dillmann (1972) administered 2,3,7,8-TCDD by gavage to 20 female mice on days 6 through 15 of gestation at doses of 0.3, 3.0, 4.5 and 9.0 µg/kg. At day 18 of gestation, extensive reabsorption was observed in the high-dose group with 6/9 litters totally resorbed. In the few surviving fetuses, there was an 81% incidence of cleft palate. At lower doses, there were 9 and 3% incidences at doses of 4.5 and 3.0 µg/kg, respectively, and no cleft palates were observed in 138 fetuses examined in the 0.3 µg/kg group. Fetal mortality was increased at the 9.0 µg/kg dose if animals were treated only on days 9 through 13; however, the incidence of cleft palate remained high at a frequency of 60%. In a series of experiments to determine the time of gestation at which 2,3,7,8-TCDD was effective in inducing cleft palate, mice were treated for a single day between days 7 and 13 of gestation with 2,3,7,8-TCDD at a dose of 45 µg/kg. A maximum number of induced cleft palates occurred when animals were treated on either day 8 or 11 of gestation; exposure to 2,3,7,8-TCDD after day 13 of gestation produced no cleft palates in the fetuses.

Courtney (1976) compared the teratogenic potential of 2,3,7,8-TCDD administered orally with 2,3,7,8-TCDD administered subcutaneously. CD-1 mice were dosed with 2,3,7,8-TCDD on days 7 through 16 of gestation at levels of 25, 50, 100, 200 or 400 µg/kg/day; the 400 µg/kg dose was not used in animals treated by subcutaneous injection. Doses of 200 or 400 µg/kg/day produced vaginal bleeding and high rates of abortion. A dose of 100 µg/kg/day was fetotoxic, resulting in decreased fetal weight and survival. Anatomic abnormalities were observed at all dose levels, with cleft palate and hydronephrotic kidneys being most common. Other abnormalities observed included hydrocephalus, open eye, edema and petechiae. Subcutaneous administration of 2,3,7,8-TCDD produced a greater teratogenic response at a lower dose than oral administration, with abnormalities observed in 87% of the fetuses following subcutaneous administration and 42% after oral administration of a dose of 25 µg/kg/day.

The effects of 2,3,7,8-TCDD on the incidence of fetal anomalies were also studied by Smith et al. (1976) in CF-1 mice. The mice were given

0.001-3.0 μg 2,3,7,8-TCDD/kg/day by gavage from day 6 through 15 of gestation. The incidence of cleft palate was found to be significantly increased in 1.0 and 3.0 μg/kg/day dose groups, and the incidence of kidney anomalies was significantly increased at 3.0 μg/kg/day. There were no observable teratogenic effects in the study at 0.1 μg/kg/day; however, some were noted at lower dose levels, although not statistically significantly elevated.

Poland and Glover (1980) compared cleft palate formation by 2,3,7,8-TCDD in the responsive C57Bl/6J, the nonresponsive DBA/2J and the hybrid B6D2F1/J strains of mice. Female mice were mated with male mice of the same genetic strain, and on day 10 of pregnancy the pregnant mice were given a single subcutaneous dose of 3.0, 10.0 or 30.0 μg/kg of 2,3,7,8-TCDD dissolved in p-dioxane or the solvent (control) alone (0.4 mℓ/kg). On day 18, the animals were killed and the number of cleft palates and resorbed fetuses was determined. At doses of 3.0 and 10.0 μg/kg of 2,3,7,8-TCDD, cleft palates (3% incidence among live fetuses) were observed only in the C57Bl/6J mice at the higher dose level. At a dose of 30 μg/kg, the incidence of cleft palates among live fetuses for the C57Bl/6J, B6D2F1/J and DBA/2J mice was 54, 13 and 2%, respectively. This study also reported that cleft palate formation was significantly higher in several other responsive mouse strains compared with nonresponsive mice. At a dose level of 30 μg/kg of 2,3,7,8-TCDD, the incidence of cleft palates among live fetuses for the responsive C57Bl/6J, A/J, BALB/cByJ and SEC/1REJ mice was 54, 73, 65 and 95%, respectively. The only responsive mouse (CBA/J) strain that was resistant to 2,3,7,8-TCDD-mediated cleft palate was also resistant to the teratogenic effects of cortisone. In contrast, the incidence of cleft palates in the nonresponsive DBA/2J, RF/J, AKR/J, SWR/J and 129/J mice was between 0 and 3% at the 30 µg/kg dose level. In a reciprocal blastocyst transfer study between 2,3,7,8-TCDD "responsive" (NMRI) and "nonresponsive" (DBA) strains of mice it has been demonstrated that 2,3,7,8-TCDD exposure (30 µg/kg bw) on day 12 of gestation developed cleft palate in 75-100% of NMRI fetuses, irrespective whether these embryo were kept in their own (NMRI) dams or transferred to DBA dams. However, none of the 2,3,7,8-TCDD exposed DBA fetuses transferred to NMRI dams or kept in their own (DBA) dams had cleft palate (D"Argy et al., 1984). These results suggest that the responsive mice, containing high levels of the Ah receptor, are highly susceptible to the effects of 2,3,7,8-TCDD in producing cleft palate, whereas the nonresponsive mice, which contain low (or 0) levels of the Ah receptor protein, are resistant to this teratogenic effect of 2,3,7,8-TCDD. These data and other results (Hassoun and Dencker, 1982) suggest that cleft palate formation elicited by 2,3,7,8-TCDD segregates with the Ah locus.

Dencker et al. (1981), Pratt (1983) and Pratt et al. (1984a,b) found an association between 2,3,7,8-TCDD-induced cleft palate and Ah activity in mice. Significant concentrations of TCDD have been detected in the placenta of pregnant TCDD-dosed mice, resulting in cleft palate induction in the fetus without any apparent effect in the dams. Sensitivities to TCDD vary among strains. The AKR strain lack Ah receptors and remain insensitive to cleft palate whereas the C57 strain possess Ah responsiveness and are sensitive to TCDD-induced cleft palate. These observations further prove that the Ah locus is the cause of strain differences in cleft palate production. It is thought that TCDD forms a complex with the Ah receptor and becomes incorporated into the chromatin. This alters the terminal differentiation of the medial epithelial cells in the palate.

**9.1.3. 2,3,7,8-TCDD Studies in Rats.** In an early study, Courtney and Moore (1971) tested the teratogenic potential of 2,3,7,8-TCDD in pregnant rats (CD) injected subcutaneously on a daily basis with 2,3,7,8-TCDD (0.5 or 2 μg/kg) in DMSO on days 6 through 15, days 9 and 10, or days 13 and 14 of gestation and examined on day 20 of gestation. Kidney malformations were observed in fetuses exposed to 2,3,7,8-TCDD. In the group exposed transplacentally at a dose of 0.5 μg/kg, 4/6 litters had fetuses with kidney malformations (average number of kidney defects/litter was 1.8). An 11 and 34% incidence of kidney anomalies occurred in groups exposed to 2,3,7,8-TCDD on days 9 and 10, and 13 and 14, respectively. In addition, six hemorrhagic GI tracts were observed in the treated group (these data were not enumerated with respect to dose); however, this was considered a primary fetotoxic effect of 2,3,7,8-TCDD and not a malformation.

2,3,7,8-TCDD was administered by gavage to groups (10-14 animals/group) of pregnant Sprague-Dawley rats at dose levels of 0, 0.03, 0.125, 0.5, 2.0 or 8.0 μg/kg/day on days 6 through 15 of gestation (Sparschu et al., 1971b). No adverse teratogenic effects were reported in fetuses exposed transplacentally at the 0.03 μg/kg level. At the 0.125 μg/kg level, three dead fetuses were reported, fetal weights were slightly depressed, and intestinal hemorrhage was noted in 18 of 127 examined fetuses. In the group given doses of 0.5 μg/kg, the number of viable fetuses was reduced, resorptions were increased, 6 dead fetuses were reported, and 36 of 99 fetuses suffered an intestinal hemorrhage. In the 2.0 μg/kg group, only 7 live fetuses were reported (occurring in only 4/11 litters), 4 having intestinal hemorrhage. Early and late resorptions were prevalent. No live fetuses, but many early resorptions, were reported in the group exposed to 8.0 μg 2,3,7,8-TCDD/kg/day. Subcutaneous edema appeared dose-related, occurring in a considerable number of fetuses from the higher dose groups. Male fetuses appeared to be more susceptible to 2,3,7,8-TCDD exposure; however, there was no significant difference in the sex ratio of live fetuses.

Khera and Ruddick (1973) tested a wide range of 2,3,7,8-TCDD doses for teratogenic and fetotoxic potential. Groups of 7-15 Wistar rats were intubated with 2,3,7,8-TCDD at doses of 0.125, 0.25, 1, 2, 4, 8 or 16 µg/kg on days 6 through 15 of gestation. At day 22 of gestation, there were no live fetuses in groups exposed to $\geq$4 µg/kg, and reduced litter size was observed in the 1 and 2 µg/kg group. Unspecified maternal toxicity was reported in all groups where there was fetal mortality. In groups exposed to 0.25-2 µg/kg, there were fetal anomalies observed as either gross or microscopic lesions consisting of subcutaneous edema of the head and neck, and hemorrhages in the intestine, brain and subcutaneous tissue. The incidences of grossly observed lesions were 0/18, 2/11, 7/12 and 11/14 in the control, 1, 1 and 2 µg/kg dose groups, respectively (the study was conducted in two parts, and the 1 µg/kg dose was repeated). With regard to the other dose levels tested, the table enumerating the results had an entry of "not done." The incidence of microscopically observed lesions for the control, 0.25, 0.5, 1, 1 and 2 µg/kg groups was 0/10, 1/33, 3/31, 3/10, 3/6 and 3/7, respectively. There were no effects of treatment observed in the 0.125 µg/kg group.

Khera and Ruddick (1973) also exposed dams to 2,3,7,8-TCDD at doses of 0.125, 0.25, 0.5 and 1 µg/kg on days 6 through 15 of gestation and allowed the dams to litter and wean the pups. In this experiment, maternal toxicity was reported in the 0.5 and 1 µg/kg group. At birth, there were fewer viable pups, and the pups had lower body weight in all but the 0.125 µg/kg group. At weaning on day 21 after birth, there were no surviving pups in the 1 μg/kg group, and 40% of the pups in the 0.5 μg/kg group did not survive. Fostering pups from dams exposed to 2,3,7,8-TCDD at 1 μg/kg onto control dams did not appreciably increase survival, while fostering control pups onto dams exposed to 2,3,7,8-TCDD did not increase pup mortality. These data suggest that poor pup survival was a result of delayed toxicity from in utero exposure to 2,3,7,8-TCDD.

Giavini et al. (1982a) assessed the effect of small doses of 2,3,7,8-TCDD administered during the preimplantation period in Sprague-Dawley rats. The animals, in groups of 20, were treated by gavage with 2,3,7,8-TCDD at doses of 0.0, 0.1, 0.5 and 2 μg/kg on days 1-3 of gestation. (The legends to the tables in this paper indicated that the low dose was 0.125 μg/kg.) At day 21 of gestation, no toxic effects were observed in the dams except for a decrease from 19.3-12.9 g in average maternal weight gain in the high dose animals as compared with controls. In the fetuses, weight was significantly reduced ($p<0.05$) in the 0.5 and 2 μg/kg groups. Malformed litters and malformation/fetuses examined were 2, 5, 5 and 6, and 2/270, 8/260, 5/255 and 8/253, respectively, in the control 0, 0.1, 0.5 and 2 μg/kg groups; however, these increases in the treated animals were not statistically significant. The anomalies observed were restricted to cystic kidney. This exposure to 2,3,7,8-TCDD early in pregnancy did not affect implantation frequency, and the decrease in fetal weight was considered a result of 2,3,7,8-TCDD delayed implantation.

In a second study, Giavini et al. (1983) administered the same doses of 2,3,7,8-TCDD (0.0, 0.125, 0.5 or 2 μg/kg) daily to 15 female CRCD rats per group by gavage in corn oil:acetone (9:1) for 2 consecutive weeks before mating. Females that did not become pregnant during three estrous cycles were necropsied to determine signs of toxicity, while pregnant animals were allowed to proceed to day 21 of gestation, at which time necropsies were performed with particular emphasis on reproductive organs and reproductive success. At the lowest dose tested (0.125 μg/kg), there were no overt clinical signs of toxicity in the dams or adverse effects in any of the fetal parameters examined. At the 0.5 and 2 μg/kg levels, average maternal weight was decreased. Also, one animal in each of these groups did not become pregnant, although necropsy did not reveal any obvious dysfunctions. The only other overt sign of toxicity was listlessness during the treatment period in the animals of the high-dose group. The only significant ($p<0.01$) fetal effect observed in the 0.5 μg/kg group was an increase in postimplantation losses from 2.9% in the control group to 10.2%. In the high-dose group, there were decreases in corpora lutea and implantations (averages of 17.6% in control and 14.9% in treated animals, and 15.5% in control and 12.0% in treated animals, respectively), and increases in both pre- and postimplantation losses of 11.7% for controls and 19.5% ($p<0.05$) in treated animals, and 2.9% in control and 30.3% ($p<0.001$) in treated animals, respectively. In addition to these signs of fetal toxicity, 9/10 litters in the high-dose group contained at least one malformed fetus as compared with 1/13, 2/13 and 2/13 in the control, 0.125 and 0.5 μg/kg groups. The predominant fetal malformations were cystic kidney and dilated renal pelvis, which have been observed in other studies in which 2,3,7,8-TCDD was administered during gestation.

The reproductive effects of 2,3,7,8-TCDD were also studied in a 3-generation study using Sprague-Dawley rats (Murray et al., 1979). Throughout the study, animals were continuously maintained on diets providing doses of 0, 0.001, 0.01 or 0.1 μg 2,3,7,8-TCDD/kg/day. The parental group ($f_0$) was maintained for 90 days on the test diets before mating. The $f_0$ rats were mated twice, producing the filial generations ($f_{1A}$ and $f_{1B}$). Selected $f_{1B}$ and $f_2$ rats were mated at ~130 days of age to produce the $f_2$ and $f_3$ litters, respectively. In later generations, the high-dose group (0.1 µg 2,3,7,8-TCDD/kg/day) was discontinued because few offspring were produced in this group. At the intermediate dose (0.01 µg/kg/day), 2,3,7,8-TCDD caused lower body weight in exposed rats of both sexes ($f_1$ and $f_2$). At the low dose, no toxic effects were discerned.

Fertility was greatly reduced in the $f_0$ generation exposed to 0.1 µg 2,3,7,8-TCDD/kg/day. At 0.01 µg 2,3,7,8-TCDD/kg/day, fertility was significantly ($p<0.05$) reduced in the $f_1$ and $f_2$ rats. Fertility in rats (of any generation) exposed to 0.001 µg 2,3,7,8-TCDD/kg/day was not different from that of control rats. Decreases in litter size were noted in the $f_{1A}$ group exposed to 0.1 µg/kg/day and the $f_2$ and $f_3$ litters exposed at 0.01 µg/kg/day. Statistically significant decreases in fetal survival throughout gestation were noted in $f_2$ and $f_3$ litters of the 0.01 µg 2,3,7,8-TCDD/kg/day exposed dams. At 0.001 µg 2,3,7,8-TCDD/kg/day, a decreased gestational survival was reported for the $f_2$ litters, but not for other generations. Decreased neonatal survival was noted among $f_{1A}$ and $f_2$ pups exposed to 0.01 µg 2,3,7,8-TCDD/kg/day, but not among $f_{1B}$ or $f_3$ pups. Postnatal body weights of the $f_2$ and $f_3$ litters at 0.01 µg 2,3,7,8-TCDD/kg/day were significantly depressed. At the low dose (0.001 µg 2,3,7,8-TCDD/kg/day), necropsy of 21-day-old pups revealed a statistically significant ($p<0.05$) increase in dilated renal pelvis in the $f_1$ generation. Subsequent generations at this dose level or any at the intermediate dose (0.01 µg 2,3,7,8-TCDD/kg/day) did not have a significant increase in this abnormality. Significantly decreased thymus weight and increased liver weight were reported in the $f_3$ generation, but not in the $f_1$ generation ($f_2$ generation data not obtained) of the intermediate dose group. Murray et al. (1979) concluded that 2,3,7,8-TCDD ingested at 0.01 or 0.1 μg/kg/day impaired reproduction among rats, and NOAELs were associated with 0.001 μg 2,3,7,8-TCDD/kg/day.

Nisbet and Paxton (1982) reevaluated the primary data of Murray et al. (1979) using different statistical methods. From this reevaluation it was concluded that 2,3,7,8-TCDD significantly reduced the gestational index, decreased fetal weight, and increased liver-to-body weight ratios and the incidence of dilated renal pelvis in both lower-dose groups. Nisbet and Paxton (1982) concluded that the dose of 0.001 μg/kg/day was not a NOAEL in this study. The FIFRA Scientific Advisory Panel has also reviewed the data from this 3-generation study and concluded that the effects observed at the 0.001 μg/kg dose were not consistent enough between the different generations to consider them treatment-related (U.S. EPA, 1979b). Although the panel considered the data suggestive of an embryotoxic effect, they concluded that 0.001 μg/kg represented a NOEL.

Crampton and Rogers (1983) 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) contaminated with 30 ppb of TCDD appears to have behaviorally teratogenic effect in Long-Evans rats at doses as low as 6 mg 2,4,5-T/kg bw administered to mother rats on day 8 of gestation.

9.1.4. 2,3,7,8-TCDD Studies in Rabbits and Ferrets. A report by Giavini et al. (1982b) describes the effects of exposure to 2,3,7,8-TCDD on fetal development in rabbits. Groups of 10-15 New Zealand rabbits were administered 2,3,7,8-TCDD by gavage at doses of 0.0, 0.1, 0.25, 0.5 and 1 μg/kg on days 6 through 15 of gestation. The dams were examined for implantation sites, resorptions and live fetuses, and the fetuses were examined for malformations on day 28 of gestation. Decreased maternal weight gain and unspecified signs of maternal toxicity occurred in dams exposed to 2,3,7,8-TCDD at doses of ≥0.25 μg/kg. At doses of 0.5 and 1 μg/kg, there were 2 and 4 deaths, respectively, among the dams. There were increases in abortions and resorptions at a dose of ≥0.25 μg/kg, and no live fetuses were detected in the high dose group. In the fetuses, the most common observation was a significant increase in extra ribs from 33.3% in the controls to 82, 66.6 and 82% in the 0.1, 0.25 and 0.5 μg/kg dose groups. Although there was no significant increase in specific soft-tissue anomalies, there was an increase from 0/87 to 3/78, 2/33 ($p<0.05$) and 2/28 ($p<0.05$) in total soft-tissue anomalies in the control, 0.1, 0.25 and 0.5 μg/kg groups. The most prevalent soft-tissue anomaly was hydronephrosis, which the authors pointed out was a common finding in rat fetuses exposed to 2,3,7,8-TCDD *in utero*. These effects were considered to be signs of embryotoxicity rather than a teratogenic effect.

In addition to the fetotoxic effects of prenatal exposure to 2,3,7,8-TCDD, Norman et al. (1978b) demonstrated that 2,3,7,8-TCDD could induce liver microsomal enzymes following *in utero* exposure. Pregnant New Zealand rabbits were given subcutaneous injections of 2,3,7,8-TCDD at a dose of 30 nmol/kg (9.6 μg/kg) on day 24 of gestation, and the livers of newborns were examined for enzyme activity within 12 hours after birth. While this treatment increased the liver cytochrome P-450 levels in the adults ~2-fold, from 1.8-3.7 nmol/mg protein, the increase in the newborns was ~5-fold, from 0.3-1.6 nmol/mg protein. SDS-polyacrylamide gel electrophoresis revealed that 2,3,7,8-TCDD induced a single form (form 6) of cytochrome P-450, and that this form was one of the two that were also induced by 2,3,7,8-TCDD in the adult liver. The identity of form 6 was confirmed by immunologic reaction and its peptide fingerprint. It was shown that induction of cytochrome P-450 in newborns resulted in levels of benzo(a)pyrene hydroxylase and 7-ethoxy-resorufin-O-deethylase activity similar to adult levels. The consequence to the newborn of these changes in the development of liver microsomal enzymes has not been established.

Muscarella et al. (1982) reported in an abstract the fetotoxic and teratogenic effects of subcutaneously administered 2,3,7,8-TCDD on ferrets. An unspecified number of animals received 1, 6, 13.5, 20, 30 or 60 μg of 2,3,7,8-TCDD/kg on day 18 of gestation or two doses given on days 18 and 20 of gestation at one-half the level of the single dose. The animals were examined on day 28, 29 or 30 of gestation and the results were reported without reference to specific experimental groups. In all test groups there were increases in fetal deaths and resorbed fetuses, along with growth retardation. Terata observed included unilateral and bilateral palatoschisi, open eyelids, anasarca and brachygnathia. The author concluded that 2,3,7,8-TCDD was a teratogen in ferrets.

9.1.5. **2,3,7,8-TCDD Studies in Nonhuman Primates.** Dougherty et al. (1975) found no evidence of teratogenicity or embryotoxicity in rhesus monkeys that were given on days 22-38 of gestation daily oral doses (in gelatin capsules) of up to 10 mg/kg/day of 2,4,5-T containing 0.05 ppm 2,3,7,8-TCDD. The 2,3,7,8-TCDD dose at the highest dose level of 2,4,5-T administered (10 mg/kg/day) would correspond to 0.5 μg 2,3,7,8-TCDD/kg/day. Palate closure in the monkey, however, occurs on gestational days 42-44 and the kidney is also a late developing organ.

Adverse effects of exposure to 2,3,7,8-TCDD on reproductive success in monkeys have also been described. Schantz et al. (1979) fed a diet containing 50 ppt 2,3,7,8-TCDD to rhesus monkeys for 20 months. Seven months into the study the female monkeys were bred to control males. There were four abortions and one stillbirth; two monkeys did not conceive even though they were mated repeatedly; and two monkeys carried their young to term. The total 2,3,7,8-TCDD intake over the 7 months was estimated by the authors to be 0.35 µg/kg, corresponding to a calculated daily dose of 0.0015 µg 2,3,7,8-TCDD/kg/day.

Allen et al. (1979) and Barsotti et al. (1979) fed adult female rhesus monkeys for 6-7 months on diets containing 50 or 500 ppt of 2,3,7,8-TCDD. These exposure levels correspond to total doses per animal at the end of 7 months of 1.8 and 11.7 µg 2,3,7,8-TCDD. Although menstrual cycles were not affected in either treatment group, 5/8 animals in the high-dose group had either decreased serum estradiol or decreased progesterone levels. Hormone levels were normal in the low dose animals. At 7 months, the females were bred with nonexposed males, and 6/8 and 3/8 females in the low- and high-dose groups, respectively, were impregnated. The animals were continued on treatment during pregnancy. Of the impregnated animals, 4/6 and 2/3 had spontaneous abortions, while the remaining impregnated animals had normal births. All of the control females (one group of 8 and another group of unspecified size) conceived and gave birth to "normal" offspring. The high dose resulted in the death of five animals between the 7th and 12th month of treatment.

McNulty (1978) treated pregnant rhesus monkeys by gastric gavage to 2,3,7,8-TCDD in a vehicle of corn oil:acetone solution. Group I animals were administered total dosage of 5 µg/kg bw (two animals), 1 µg/kg bw (four animals) and 0.2 µg/kg bw (four animals) in nine divided doses, 3 times/week during weeks 4, 5 and 6 (days 20 through 40) after conception. Group II, consisting of 12 animals, received single doses of 1 µg/kg bw of

2,3,7,8-TCDD on days 25, 39, 35 and 40 after conception. Three animals were exposed in each of these 4 days. The vehicle control group, consisting of 11 animals, was treated with corn oil:acetone only, on the same schedule as Group I animals. Both of the females that received the highest dose (5 μg/kg) had fetal losses. In the next lower-dosed animals (1 μg/kg in both groups), 12 of 16 females had fetal losses; and in the lowest-dosed animals (0.2 μg/kg in Group I), one abortion occurred in four pregnancies. Maternal toxicity was observed in many of these treated females. The difference in frequency of fetal loss between all pregnant animals given 1 μg/kg and the rate of historical abortion in the author's breeding colony was found to be significant. The author concluded that short exposure to 1 μg/kg bw of 2,3,7,8-TCDD during early pregnancy results in fetal loss in rhesus monkeys. In a recent report, McNulty (1984) reveals that he failed to detect any malformations in the fetus but observed widespread maternal toxicity and fetocidal effects in monkeys as a result of intragastric exposure to 2,3,7,8-TCDD.

9.1.6. Studies in Chickens. The effects of 2,3,7,8-TCDD on the development of the heart in chicken embryos was studied by Cheung et al. (1981) as a consequence of the known induction of hydropericardium by 2,3,7,8-TCDD in adult chickens and the relation between changes in hemodynamics and cardiovascular malformation. Groups of at least 20 White-Leghorn eggs were injected with 2,3,7,8-TCDD in acetone:corn oil (0.5:9.5 v/v) on day zero of embryo development. Administered doses ranged from 0.009-77.5 pmol/egg (0.00029-2.5x$10^{-2}$ μg/egg) in 5 μℓ. The embryos were examined on day 14 of development. A dose-related increase in cardiovascular malformations was observed with 1 pmol/egg resulting in malformations in 50% of the embryos. Increases in all types of malformations (ventricular septal defect, aortic arch anomaly, aortic arch anomaly and ventricular septal defect, and conotruncal malformations) occurred. Hydropericardium was observed in some embryos (not enumerated), but it could not be concluded that this was the cause of the cardiovascular malformations. Malformed legs and crossed beaks associated with micropthalmia was observed in treated embryos, however, the incidence, 7/284 and 2/284, respectively, was low.

9.1.7. Studies of the Teratogenic and Reproductive Effects of HxCDD. In addition to 2,3,7,8-TCDD, the teratogenic potential of a related chlorinated dibenzo-p-dioxin compound, HxCDD (congeners not specified), has been investigated in rats. Pregnant Sprague-Dawley rats were treated by gavage with 0.1, 1.0, 10 or 100 μg HxCDD/kg/day on days 6-15 of gestation (Schwetz et al., 1973). Treatment with high levels of HxCDD (10 and 100 μg/kg) was highly lethal to fetuses during late gestation. There was a significant dose-related increase in late resorptions from 0% (at 0.1 μg/kg/day) to 79% (at 100 μg/kg/day). Decreases in the weight and length of surviving fetuses were due to HxCDD. The incidences of cleft palate, subcutaneous edema, malformed vertebrae and split sternebrae were significantly increased in fetuses of rats treated with 100 μg HxCDD/kg/day. No increase in fetal anomalies was noted in fetuses exposed to 0.1 μg HxCDD/kg, and only subcutaneous edema was more prevalent in groups exposed at 1 or 10 μg HxCDD/kg/day when compared with controls.

Pertinent information regarding the teratogenicity or reproductive effects of PeCDDs was not located in the available literature.

## 9.2. STUDIES ON HUMAN POPULATIONS

A positive association between 2,4,5-T exposures and increases in birth defects or abortions has been reported in human populations in Oregon (U.S. EPA, 1979c), New Zealand (Hanify et al., 1981), and Australia (Field and

Kerr, 1979). A lack of any such association has been reported in human populations in Arkansas (Nelson et al., 1979), Hungary (Thomas, 1980b), New Zealand (Dept. of Health, New Zealand, 1980; McQueen et al., 1977), and Australia (Aldred, 1978). Almost all of the reports are geographic correlation studies, and because of the uncertainties inherent in this type of epidemiologic investigation, as well as the difficulties in distinguishing the effects of 2,4,5-T from those of 2,3,7,8-TCDD contamination, none of the reportedly positive associations unequivocally identify either 2,4,5-T or 2,3,7,8-TCDD as the causative agent. Similarly, the reportedly negative associations do not rule out 2,4,5-T or 2,3,7,8-TCDD as potential teratogens or abortifacients in humans.

Based on a report of a high incidence of abortions in a small group of women living around Alsea, Oregon, who may have been exposed to the herbicide 2,4,5-T from aerial spraying (Smith, 1979), the U.S. EPA (1979c) initiated a study, often referred to as the "Alsea II study," to determine if spontaneous abortion rates differed between the exposed and unexposed populations, if spontaneous abortion rates evidenced seasonal variation in these two groups, and if such seasonal variations were associated with 2,4,5-T spray application.

The Spontaneous Abortion Rate Index, as defined by the U.S. EPA, is "basically the ratio of the number of hospitalized spontaneous abortions to the number of births corresponding to the spontaneous abortions, based on the residence zip code of the women contributing to each event." Upon completion of the study, the U.S. EPA concluded that (1) the 1972-1977 Spontaneous Abortion Rate Index for the study area was significantly higher than in the Rural Control Area or the Urban area; (2) there was a statistically significant seasonal cycle in the abortion index in each of the areas with a period of ~4 months. In particular there was an outstanding peak in the study area in June; and (3) there was a statistically significant correlation between the Spontaneous Abortion Rate Index and spray patterns in the study area when a lag-time of 2 or 3 months was included. The U.S. EPA concluded, however, that "This analysis is a correlational analysis, and correlation does not necessarily mean causation."

Milby et al. (1980), citing three critiques of the Alsea II study (not published in the open literature), state that the statistical method and basic design of the Alsea II study were sufficiently flawed to make this study of no use in human risk assessment. The Alsea II study has also been reviewed by a panel of scientists who, in a published report of their meeting, also concluded that the basic design of the study was inadequate to demonstrate either an effect or absence of an effect of exposure to 2,4,5-T (Coulston and Olajos, 1980). The major inadequacies of the study were that the data collection methods were likely to result in the underestimation of abortions, particularly in the urban area (the incidence of abortions in all three groups was within the expected background rate of 8-15%); only a small part of the area from which the exposed subjects were selected was actually sprayed with 2,4,5-T, and the study was not controlled for other factors such as age, smoking habits and alcohol consumption, which may affect the spontaneous abortion rate. Based on a new report by Smith (1979), the U.S. EPA is attempting or has attempted to correlate 2,3,7,8-TCDD levels in the affected areas with the observed rate of abortion. No published reports have been located on the outcome of this effort.

Nelson et al. (1979) noted a general increase in the reported incidence of facial cleft in both high and low exposure groups in Arkansas from 1948-1974. In this study, exposure estimates were based on average rice production in different areas of Arkansas, and the incidence of cleft palate was determined by screening birth certificates and checking records of the Crippled Children's Services. No consistent exposure/effect correlations were noted, and the general increase with time in the incidence of facial clefts was attributed to better reporting procedures; however, there does not have to be a direct correspondence of malformations in human beings and experimental animals.

Of the four reports available from New Zealand (Dept. of Health, New Zealand, 1980; McQueen et al., 1977; Hanify et al., 1981; Smith et al., 1982a), the report by the Department of Health is essentially anecdotal, involving two women who gave birth to malformed children (one with an atrial septal defect and a malformation of the tricuspid valve of the heart, and the other with biliary atresia). In both cases, exposure to 2,4,5-T could not be ruled out. Based on an analysis of spraying records, the time course of the pregnancies and plant damage near the women's homes, however, the Department of Health, New Zealand (1980) concluded that there was insufficient evidence to implicate 2,4,5-T spraying as a causative factor. Even if the spraying had been implicated, a lack of information on 2,3,7,8-TCDD levels in the spray and the absence of any monitoring data on 2,4,5-T or 2,3,7,8-TCDD would limit the usefulness of this report.

The study by McQueen et al. (1977) is not published in the open literature but is summarized by Milby et al. (1980). According to the summary, McQueen et al. (1977) "...examined the epidemiology of neural-tube defects in three areas in New Zealand and concluded 'there is no evidence to implicate 2,4,5-T as a causal factor in human birth defects.'" No additional details are provided.

Hanify et al. (1981) performed an epidemiologic study in Northland, New Zealand, in areas where spraying of 2,4,5-T was done by various companies for a number of years. The rate of birth defects was obtained from an examination of hospital records in seven nonoverlapping areas on a monthly basis over a period extending from 1959-1977. The rate of birth defects from 1959-1965 represented the rate for a nonexposed population since this was prior to the use of 2,4,5-T, while the incidence of birth defects from 1972-1976 represented the rate for the exposed population. During the time of the survey there were 37,751 births, 436 stillbirths, 264 deaths shortly after birth, and 510 congenital anomalies. Three categories of birth defects, heart abnormalities, hypospadias and epispadias, and talipes, had elevated rate ratios of >1 ($p=0.05$) in comparisons between the exposed (1972-1976) and control (1959-1965) populations. Exposure estimates were made for the seven areas and for different years using company records of aerial spraying and a model that factored in assumed fractional removal rates/month (this factor was assumed to be either 1.0 or 0.25). Comparisons of the rate of specific malformations with exposure demonstrated a statistically significant association between the occurrence of talipes and exposure when the fractional removal rate was assumed to be 0.25. There was, however, no statistically significant association where 1.0 was used as the fractional removal rate.

Smith et al. (1982a) investigated the outcome of pregnancy in families of professional 2,4,5-T applicators and agricultural contractors in New Zealand. Agricultural contractors were chosen as the control population since both sprayers and contractors were of the same economic group with similar outdoor occupations. The survey was conducted by mail with 89% of the chemical applicators responding and 83% of the agricultural contractors responding to questions asking whether they used 2,4,5-T and its temporal relationship to reproductive histories regarding birth, miscarriages, stillbirths and congenital defects. The relative risks of congenital defects and miscarriages were 1.19 (0.58-2.45% confidence limits) and 0.89 (0.61-1.30% confidence limits) for the wives of chemical sprayers as compared with the wives of agricultural contractors. These data indicate that exposure of fathers and mothers (i.e., while cleaning clothes) had no effect on the outcome of pregnancy. Biases that may have affected the results, such as the age of the mother at childbirth, smoking habits and birth to Maori parents were investigated and eliminated as possible confounders.

The two reports from Australia (Aldred, 1978; Field and Kerr, 1979) also present apparently conflicting results. The report by Aldred (1978) is not published in the open literature, but the following summary is taken from Milby et al. (1980): "The report concluded that birth defects in a group of babies born in the [Yarram] district in 1974 and 1976 could not be attributed to exposure to 2,4,5-T or 2,4-D." Additional details that might be useful in assessing the rationale for this statement are not provided in the summary. The report by Field and Kerr (1979) plotted the incidence of neural-tube defects (anencephaly and meningomyelocele) in New South Wales, Australia, over the years 1965-1975, and the usage of 2,4,5-T in all of Australia during the previous years. The authors noted a decrease in the incidence of neural-tube defects expected on the basis of the plotted line in 1975 and 1976, when Australia instituted monitoring of 2,4,5-T to ensure a 2,3,7,8-TCDD level <0.1 ppm. The data were not tested for significance; although Field and Kerr (1979) indicate that they consider the epidemiological data on neural-tube defects to be "relatively complete," they do not comment on the increasing incidence of neural-tube defects during the time period of this study and whether or not an increase in the thoroughness of reporting neural-tube defects could have contributed to the apparent correlation of 2,4,5-T exposure with these defects. A replotting of the data suggests that the incidence of cleft palate correlates better with 2,4,5-T usage than with time. Nonetheless, the appropriateness of correlating 2,4,5-T usage in all of Australia with the incidence of defects in one area of Australia is questionable.

Thomas (1980b) used an approach similar to that of Field and Kerr (1979) on data from Hungary. One major difference, however, is that Thomas (1980b) compared the incidence of stillbirths, cleft lip, cleft palate, spina bifida, anencephalus and cystic kidney disease in all of Hungary between 1976 and 1980 with 2,4,5-T use in 1975 in all of Hungary. Because Hungary requires compulsory notification of malformations diagnosed from birth to age 1 year, because a relatively large percentage (55%) of the Hungarian population lives in rural areas where 2,4,5-T exposure may be expected to be greatest, and because annual use of 2,4,5-T in Hungary had risen from 46,000 kg in 1969 to 1,200,000 kg in 1975, Thomas (1980b) considered Hungary to be "...probably the best country in which to examine possible health effects of this herbicide." All indices of birth defect rates decreased or remained stable over the period of study.

In addition to contamination of 2,4,5-T being a potential source of 2,3,7,8-TCDD exposure, 2,3,7,8-TCDD is also an inadvertent contaminant of 2,4,5-trichlorophenol (TCP). Chronic exposure to 2,3,7,8-TCDD may occur during the manufacture of TCP and high level acute exposure to 2,3,7,8-TCDD has occurred after an accident in July, 1976 at the ICMESA TCP chemical factory in Seveso, Italy (Bonaccorsi et al., 1978). In this accident, the reaction used to produce TCP became uncontrolled, producing conditions favorable for 2,3,7,8-TCDD formation before venting the contents of the chemical reactor into the atmosphere. The resulting cloud of chemicals settled over a heavily populated area. Although the amount of 2,3,7,8-TCDD released was not known, the reported cases of chloracne, a symptom of acute exposure to 2,3,7,8-TCDD, indicated that exposure to 2,3,7,8-TCDD had occurred. Some preliminary results are available from epidemiologic studies of reproductive events in the inhabitants of Seveso, and recently a study has become available on the reproductive history of men employed in the chemical manufacturing industry with possible chronic exposure to 2,3,7,8-TCDD (Townsend et al., 1982).

Epidemiologic studies to determine the reproductive effects in individuals exposed to 2,3,7,8-TCDD and TCP following the accidental contamination of a populated area around Seveso, Italy, are not completed. The incidence of spontaneous abortions occurring between March 1976 and January 1978 have been reported for inhabitants in the area around Seveso by Bonaccorsi et al. (1978), Reggiani (1980) and Bisanti et al. (1980). The spontaneous abortion rate in the contaminated area for the three trimesters following the accident was 13.1, 11.0 and 13.05%, which was similar to the worldwide 15-20% frequency of spontaneous abortion. Subdividing the contaminated area into highly, moderately, and least contaminated, and examining the rates for each area individually, also failed to demonstrate any change in the spontaneous abortion rate. The incidence rates of malformations also were examined; however, the numbers were too few for meaningful assessment. There are several inadequacies in these studies that might make them insensitive in detecting reproductive effects. The authors noted that there are many difficulties in interpreting these data. Adequate data on the incidence rates of spontaneous abortions and birth defects were not adequately available for the region before the accident as a result of suspected underreporting. There was inadequate reporting even after the accident because of political turmoil with regard to the management of health services. Also, an unknown number of pregnancies were surgically aborted for fear of 2,3,7,8-TCDD-induced birth defects. In a recent review of the progress of epidemiologic investigations of the Seveso accident, Tognoni and Bonaccorsi (1982) indicated that the data on spontaneous abortions and malformation rates still needed verification, and that these data were too preliminary to allow for conclusions.

Townsend et al. (1982) investigated the reproductive history of wives of employees potentially exposed to 2,3,7,8-TCDD during chlorophenol production in Midland, MI. A total of 930 potentially exposed males were identified who had worked for $\geq 1$ month between January 1939, and December 1975, in a job with potential 2,3,7,8-TCDD exposure. Exposure estimates of low, moderate and high were made by an industrial hygienist primarily from job description and surface contamination data; however, the high potential exposure group was reserved for process workers during 1963-1964 when changes in operations resulted in a number of cases of chloracne. The control population was an equal number of male employees not involved in any process that might involve exposure to 2,3,7,8-TCDD and matched for date of hire. In these groups, 586 wives were identified and 370 agreed to participate as the exposed group, while 345 wives of a potential control group of 559 agreed to participate. After identification of the participants, a personal interview was conducted with the wives to determine pregnancy outcome. Of the total of 737 conceptions in the exposed category and 1785 conceptions in the control category (conceptions that occurred in the exposed group before work records indicating potential exposure to 2,3,7,8-TCDD were placed in the control group), there was no statistically significant increase in spontaneous abortions, stillbirths, infant deaths or selected congenital malformations. Sample sizes were too small to provide meaningful data if the populations were subdivided by extent of exposure. The authors suggested that many confounding factors could account for these negative results, such as the inappropriate selection of the populations, the use of "exposed" persons in both exposed and control groups, unidentified covariables and low power; however, it was asserted that these results were consistent with animal data, which report that paternal exposure to 2,3,7,8-TCDD does not affect the conceptus.

Poole (1983), in testimony before the House Committee on Science and Technology, described a reanalysis of the primary data used by Townsend et al. (1982). In this reanalysis, the relative risk of cleft palate and cleft lip were reported to be 1.9 (90% confidence intervals of 1.0-3.6) in the years 1971-1974 for both the control and exposed groups (the comparison population was not described). At the same House Committee hearing, Houk (1983) presented data from the Birth Defect Monitoring Program of the Centers for Disease Control on the yearly rate of cleft palate alone or cleft lip with or without cleft palate for births in Midland County, Michigan (the site of Dow's chlorophenol production facility) during the years 1970-1981. The data indicated an increased rate for these defects of between 50 and 100% in the years 1971-1975, with the rate returning to normal from 1976-1981. The observed increase was statistically significant if the rates for cleft palate alone and cleft lip with or without cleft palate were combined; however, it was the opinion of Houk (1983) that these defects should not be combined since the causal mechanism may be different. The Michigan Department of Public Health (1983a) also reported these results and, in addition, demonstrated that the same results occurred if the comparison was made with other counties in Michigan as well as with the general population of the United States. It was noted in this report that "runs" of increases in oral cleft for successive years have occurred in six other counties with no obvious chemical exposure. The Michigan Department of Public Health (1983a) interpreted the data to indicate that a more detailed case control study was necessary to determine if any common factors may exist, such as exposure to chemicals contaminated with 2,3,7,8-TCDD.

A similar but limited study of the reproductive history of the wives of employees of the Long Island Railroad was performed by Honchar for NIOSH (1982). The employees were concerned about the use of 2,4,5-T for maintenance along the right-of-way. There were 170 live births as indicated by union files during the study period from 1975-1979. For each birth, insurance claims were reviewed to determine any health problems during the first year of life. The incidence of major birth defects was underrepresented in the study population when compared with data from the Metropolitan Atlanta Congenital Defects Program (3 observed and 3.81 expected). Some minor health problems (i.e., tear duct obstruction) were elevated; however, the authors considered this to have resulted from diagnostic bias. It was concluded that no association between birth defects and exposure to 2,4,5-T was demonstrated in this study.

To test any possible association between birth defects and exposure to Agent Orange in Vietnam veterans, Erickson et al. (1984) conducted a case-control study on newborns with various types of congenital defects in the metropolitan Atlanta area during the years 1968 through 1980. Though most of the Vietnam veterans received from the Army Agent Orange Task Force an estimated opportunity index score regarding their exposure to Agent Orange, 25% of the Vietnam veterans interviewed in this study felt that they were exposed and approximately an equal proportion did not know if they were exposed to Agent Orange. Increased estimated risks for fathering babies with 1) spina bifida, 2) cleft lip with or without cleft palate and 3) certain tumors were found in this study. However, the authors concluded that "Vietnam veterans who had greater estimated opportunity for Agent Orange exposure did not seem to be at a greater risk for fathering babies with all types of defects combined" (Erickson et al., 1984).

## 9.3. OTHER REPRODUCTIVE EFFECTS

The effects of a mixture of 2,4,5-T, 2,4-D and 2,3,7,8-TCDD (simulated Agent Orange; however, the free acids were used rather than butyl esters to eliminate problems of volatility) on the fertility and reproductive capacities of male C57Bl/6 mice were studied by Lamb et al. (1980, 1981a). Groups of 25 mice were treated with dietary levels of the three compounds so that the daily doses/kg bw were 40 mg each of 2,4,5-T and 2,4-D, and 2.4 μg of 2,3,7,8-TCDD (Group II); 40 mg each of 2,4,5-T and 2,4-D and 0.16 μg of 2,3,7,8-TCDD (Group III); or 20 mg each of 2,4,5-T and 2,4-D and 1.2 μg of 2,3,7,8-TCDD (Group IV). A vehicle control group (Group I) was given a diet containing 2% corn oil. An 8-week exposure period was followed by an 8-week observation period during which fertility and reproductive assessments were conducted. Sperm concentrations, sperm motility and sperm abnormalities were evaluated. In addition, the males were mated with virgin females (3 females/week for 8 post-treatment weeks) to assess mating frequency, average fertility, percent implantations and resorptions, and percent fetal malformations. There was no significant decrease in any of the parameters used as a measure of fertility and reproductive capacity in any groups of treated mice when compared with controls. Lamb et al. (1981b), in a further report of this work, indicated that germ cell toxicity was not apparent and survival of offspring of exposed mice was unaffected. No external, visceral or skeletal terata were noted in offspring whose sires were exposed to the phenoxy acids/2,3,7,8-TCDD mixture in this study. The only effects noted were dose-related decreases in body weight in the treated males, and these effects were reversed when treatment was terminated.

## 9.4. SUMMARY

2,3,7,8-TCDD has been demonstrated to be teratogenic in all strains of mice tested. The most common malformations observed are cleft palate and kidney anomalies; however, other malformations have been observed occasionally. With an MED of 1 μg/kg/day for mice, 2,3,7,8-TCDD is the most potent teratogen known. At higher doses, 2,3,7,8-TCDD has a marked fetotoxic effect, as measured by decreased fetal weight and increased fetal toxicity. Hemorrhagic GI tract has been associated with 2,3,7,8-TCDD fetal toxicity.

In rats, it has also been observed that 2,3,7,8-TCDD produced teratogenic and fetotoxic responses in all strains tested. In this species, the most common fetal anomalies observed were edema, hemorrhage and malformation of the kidney with effects observed at doses of $\geq 0.1$ μg/kg/day. In addition, there is some evidence that 2,3,7,8-TCDD can induce microsomal enzymes in the fetus exposed _in utero_, and this induction is accompanied by damage to the fine structure of the liver cell; however, other reports indicate that enzyme induction occurs only after birth following exposure to 2,3,7,8-TCDD through the mother's milk. As in mice, hemorrhagic GI tracts have been observed in rat fetuses exposed _in utero_ to 2,3,7,8-TCDD.

Rabbits and monkeys are also susceptible to the fetotoxic effects of 2,3,7,8-TCDD; however, the studies of these species have been too limited to clearly evaluate a teratogenic response or define a threshold dose for fetotoxicity.

A number of studies, mostly correlation studies, have been conducted on groups of persons exposed to 2,3,7,8-TCDD as a contaminant of the herbicides 2,4,5-T or the chemical of TCP. Although some studies have shown a positive association between exposure to 2,4,5-T and birth defects or abortions, other studies have not. In investigations concerning potential exposure to 2,3,7,8-TCDD through the manufacture of TCP, there has been no positive substantiated association between exposure and reproductive difficulties. In these studies, exposure was always mixed, with 2,3,7,8-TCDD being only a minor component. Hence, it is not possible to attribute with certainty any positive finding to 2,3,7,8-TCDD. It is also possible, since levels of 2,3,7,8-TCDD contamination of 2,4,5-T and TCP were only estimated, that the negative results reflect the exposure was too low or the study designs too insensitive to elicit a detectable response. From an extensive review of dioxin-induced animal and human reproductive toxicity data by Mattison et al. (1984) and another review of 15 reports dealing with human exposure to dioxins and reproductive effects by Hatch (1984), it can be concluded that epidemiologic observations from well designed studies are warranted before deriving any conclusion on dioxin-induced reproductive toxicity in humans. Although the evidence from human studies is insufficient to prove 2,3,7,8-TCDD is teratogenic, the animal data clearly indicate teratogenic or fetotoxic effects in all animal species tested.

# 10. MUTAGENICITY AND OTHER INDICATIONS OF GENOTOXICITY

## 10.1. RELEVANT STUDIES

10.1.1. Assays in Microorganisms. Short-term *in vitro* test systems have been developed to assess the biologic, toxic and genotoxic effects of chemicals. These assays have proven to be useful indicators of potential activity of diverse industrial chemicals, a broad range of drugs and xenobiotics, carcinogens and crude environmental extracts. The most widely used short-term test system, the Ames test for bacterial mutagenesis, employs several strains of *Salmonella typhimurium* that are highly susceptible to the effects of mutagenic chemicals. Despite the obvious utility of the Ames test and related short-term assays, their predictive capabilities (i.e., the correlation between bacterial mutagenicity and carcinogenicity) have not been fully assessed (Bartsch et al., 1982).

Mutagenicity assays in microorganisms have been used to assess the genotoxic effects of 2,3,7,8-TCDD; however, the results of most of these assays have indicated little potential for mutagenic effects (Table 10-1).

Hussain et al. (1972) exposed *S. typhimurium* histidine-dependent strains TA1530 and TA1532 in liquid suspension to 2,3,7,8-TCDD followed by plating into selective medium to observe reversion to prototypes. No increase in the reversion rate was observed with strain TA1530 at exposure levels of 1 and 10 μg/mℓ. These exposures resulted in cell survivals of 90 and <1%, respectively. In strain TA1532, increased reversion frequency was not observed at 2,3,7,8-TCDD concentrations of <2-3 μg/mℓ, which resulted in a 0-50% decrease in survival; however, at 2,3,7,8-TCDD levels that resulted in a 99% decrease in survival, there was an increased number of revertant colonies/surviving cells. This positive response is questionable because of the extremely high toxicity observed. The dose levels were not specified.

TABLE 10-1

The Results of Mutagenicity Assays for 2,3,7,8-TCDD in Salmonella typhimurium

| Type of Assay | Strains of Salmonella typhimurium | | | | | | | | | | | | | | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | S-9 | TA98 | TA1530 | TA1535 | TA1537 | TA1538 | TA1532 | TA1950 | TA1975 | TA1978 | G46 | TA100 | TA1531 | TA1534 | |
| Spot test | +/- | NT | NT | 0 | 0 | 0 | 0 | NT | NT | NT | NT | NT | NT | NT | McCann, 1978 |
| Plate incorporation | +/- | NT | NT | 0 | 0 | 0 | 0 | NT | NT | NT | NT | NT | NT | NT | McCann, 1978 |
| Plate incorporation* | +/- | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | NT | NT | Gilbert et al., 1980 |
| Fluctuation test | +/- | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | NT | NT | Gilbert et al., 1980 |
| Spot test | - | NT | 0 | NT | NT | NT | + | NT | NT | NT | 0 | NT | QR | QR | Seiler, 1973 |
| Plate incorporation | + | 0 | NT | 0 | 0 | 0 | NT | NT | NT | NT | NT | 0 | NT | NT | Geiger and Neal, 1981 |
| Plate incorporation | - | NT | NT | NT | 0 | NT | NT | NT | NT | NT | NT | NT | NT | NT | Geiger and Neal, 1981 |
| Suspension assay | - | NT | 0 | NT | NT | NT | QR | NT | NT | NT | NT | NT | NT | NT | Hussain et al., 1972 |
| Suspension assay | +/- | 0 | NT | 0 | 0 | NT | NT | NT | NT | NT | NT | 0 | NT | NT | Mortelmans et al., 1984 |

*The assay was performed under both aerobic and anaerobic conditions.

NT = Not tested; QR = Questionable response; 0 = Negative response; + = Positive response

The source of the 2,3,7,8-TCDD sample studied in this paper was the Food and Drug Administration, and its reported purity was 99%. Also, Seiler (1973) observed a positive mutagenic response in a spot test of 2,3,7,8-TCDD performed in the absence of a metabolic activation system. However, the purity of the sample studied was not provided. In tester strains G46 and TA1530, the ratio of revertants/$10^8$ cells in the treated plates divided by spontaneous revertants/$10^8$ cells was <1. In strains TA1531 and TA1534, the ratio was between 1 and 2, which was considered a "doubtful" mutagenic response, while in strain TA1532, the ratio was >10. There was no mention of the 2,3,7,8-TCDD levels tested in this assay. The positive controls, diethylsulfate, 2-aminopurine and 2-aminofluorene, produced ratios of 2 to 5, <1 and 5 to 10, respectively, in strain TA1532. In both the study by Hussain et al. (1972) and the study by Seiler (1973), 2,3,7,8-TCDD produced a positive mutagenic response only in the S. typhimurium strain TA1532, which is sensitive to frameshift mutagens.

Hussain et al. (1972) also performed a mutagenicity test of 2,3,7,8-TCDD in two other microbial test systems. A positive response was observed in Escherichia coli Sd-4 as indicated by a reversion to streptomycin independence. In this assay, cells were treated in suspension for 1 hour with 2,3,7,8-TCDD at 0.5-4 µg/mℓ. The greatest mutation frequency (256 mutants x $10^{-8}$, as compared with the control frequency of 2.2 mutants x $10^{-8}$) occurred at a dose level of 2 µg/mℓ. The absolute number of colonies/plate was 7 for the control and 46 for the treated plate. The dose of 2 µg/mℓ caused an 89% decrease in cell survival. A duplicate sample resulted in an 82% decrease in survival and a mutation frequency of $34 \times 10^{-8}$. These results indicate that the reproducibility of the assay may not have been perfect, but both results are well above the control value of

$2.2 \times 10^{-8}$. A dose-response relationship was not observed, indicating that the results at 2 μg/mℓ are only suggestive of a positive response. In addition, the positive results were obtained at a concentration of 2,3,7,8-TCDD (2 μg/mℓ) that was well above solubility in water (0.2 μg/ℓ), which also casts doubt on the significance of the positive result. In the second test system, the ability of 2,3,7,8-TCDD to increase prophage induction in *E. coli* K-39 cells was examined. The vehicle control, DMSO, inhibited prophage induction as compared with the untreated controls, while the most effective dose level of 2,3,7,8-TCDD (0.5 μg/mℓ) resulted in an increased prophage induction as compared with the vehicle control but not as compared with the untreated controls. Hussain et al. (1972) concluded that 2,3,7,8-TCDD was capable of causing increases in the reverse mutation rate in *E. coli* Sd-4 and that 2,3,7,8-TCDD had a weak ability to induce prophage in *E. coli* K-39 cells.

The studies that followed these two early reports of Hussain et al. (1972) and Seiler (1973) failed to detect mutagenic activity of 2,3,7,8-TCDD in *S. typhimurium*. Wassom et al. (1978) cited a personal communication from McCann (1978), which reported that 2,3,7,8-TCDD was inactive in both the spot test and plate incorporation assay with *S. typhimurium* strains TA1532, TA1535, TA1537 and TA1538. Doses and other experimental protocols were not mentioned except that the tests were performed both with and without metabolic activation. Gilbert et al. (1980) reported that 2,3,7,8-TCDD gave "substantially negative results" with *S. typhimurium* strains TA98, TA100, TA1530, TA1535, TA1537, TA1538, G46, TA1532, TA1950, TA1975 and TA1978. Both the standard plate incorporation assay and the bacterial fluctuation test were used, and both were performed with and without S-9 prepared from the livers of Aroclor 1254 pretreated rats. In the plate incorporation assay, the test compound was tested at 1-2000 μg/plate under both aerobic and anaerobic conditions. Details were not provided for the fluctuation assay. It is difficult to assess possible reasons for the conflicting results between the earlier studies and these later mutagenicity assays, since information on experimental conditions was limited in the negative studies.

In an attempt to resolve the conflicting results and observe a mutagenic response, Geiger and Neal (1981) tested 2,3,7,8-TCDD in the standard plate incorporation assay using S-9 prepared from different sources. In order to maximize the amount of compound tested, dioxane, a better solvent for 2,3,7,8-TCDD than the commonly employed DMSO, was used. Even with the use of dioxane, the limited solubility of 2,3,7,8-TCDD allowed only 20 μg/plate to be tested, a dose that was shown to be nontoxic to the cells. The S-9 used in these assays was prepared from the livers of Aroclor 1254 pretreated male Sprague-Dawley rats and male Golden Syrian hamsters, and from 2,3,7,8-TCDD induced male hamsters. In all assays at 2,3,7,8-TCDD concentrations of 0.2, 2, 5 or 20 μg/plate, and regardless of the source of the S-9, there was no observed mutagenic response. In further attempts to duplicate the previous positive results, Geiger and Neal (1981) tested the same concentrations of 2,3,7,8-TCDD in strain TA1537, a more sensitive direct descendent of strain TA1532, for mutagenic activity in the absence of S-9. Again, no increase in the number of revertants was observed. In assays either with or without S-9, positive controls had predictable increases in the number of revertant colonies. The authors concluded that 2,3,7,8-TCDD was not active under the conditions of this assay; however, testing at higher concentrations may elicit a positive response. It was also noted that many other polychlorinated aromatic compounds are not mutagenic in the Ames test, even though there is positive evidence of carcinogenicity.

The National Toxicology Program (NTP) provided data on 2,3,7,8-TCDD from four assay systems: the S. typhimurium (strains TA98, TA100, TA1535 and TA1537) histidine reversion assay, the sex-linked recessive lethal test in Drosophila, and cytogenetic studies (sister chromatid exchange and chromosome aberrations) in Chinese hamster ovary cells. Negative results were obtained in all of these assays (Mortelmans et al., 1984; Zimmering et al., 1985; NTP, 1985).

Mutagenic effects of 2,3,7,8-TCDD in yeast were observed by Bronzetti et al. (1983). Positive results for reversion and gene conversion were obtained in vitro and in the host-mediated assay. The in vitro experiments yielded small dose-related increases in $trp^+$ convertants and $ilv^+$ revertants. An S10 metabolic activation system was required. Exposure of the yeast to 2,3,7,8-TCDD at the highest level tested (10 μg/mℓ) resulted in 16% survival and yielded 4-fold increases in reversion and gene conversion.

In the host-mediated assay, male mice were exposed to 25 μg of 2,3,7,8-TCDD/kg (Bronzetti et al., 1983). After 5, 10, 20 or 30 days, 0.2 mℓ of a yeast culture ($4 \times 10^8$ cells) was instilled retroorbitally. Four hours later, the liver and kidneys were removed and the yeast cells in these organs were assayed for mutagenic responses. Increases (4- to 6-fold) in reversion and gene conversion were observed in yeast cells obtained from the livers and kidneys. The toxic response of the animals to an exposure of 25 μg/kg was not described in this report. The positive results described in this paper suggest that 2,3,7,8-TCDD is mutagenic in yeast, but more definitive studies are needed before a firm conclusion can be drawn.

Hay (1982) has found that 2,3,7,8-TCDD dissolved in DMSO transformed baby hamster kidney cells (BHK) in vitro. The dioxin isomers 2,8-dichloroand 1,3,7-trichlorodibenzo-p-dioxin also transformed BHK cells, but the response was weak. The unchlorinated dibenzo-p-dioxin and the fully chlorinated octachlorodibenzo-p-dioxin were both negative in the BHK assay (i.e., there was no cell transformation).

Abernethy et al. (1985) failed to transform C3H/10T$_{1/2}$ cells in culture by single treatments with 0.06 mM to 5 μ dosage of 2.3.7.8-TCDD or initiate transformation in these treated cells by subsequent exposure with tumor promoter 12-O-tetradecanoylpharbol-13-acetetate (TPA). However, these authors could transform C3H/10 T$_{1/2}$ cells _in_ _vitro_ by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and this transformation could be enhanced by subsequent treatment with low concentration ($\geq$ 4 pM) of 2,3,7,8-TCDD. Maximum enhancement was observed at a concentration of 40 pM of 2.3.7.8-TCDD. This study indicates that 2,3,7,8-TCDD induced promotional activities can be observed in C3H/10 T$_{1/2}$ cells in cultured.

Rogers et al. (1982) reported that 2,3,7,8-TCDD induced mutations in the excess thymidine, thioguanine and methotrexate selective systems in L5178Y mouse lymphoma cells in culture. However, no significant mutation was noted in ouabin or cytosine arabinoside selective systems.

10.1.2. Interactions with Nucleic Acids. _In_ _vitro_ reactions of 2,3,7,8-TCDD with bacteriophage QB RNA were evaluated by Kondorosi et al. (1973). Active RNA was purified from QB phage followed by incubation for 1 hour at 37°C with 0.0, 0.2, 2.0 or 4.0 μg/mℓ of 2,3,7,8-TCDD. At all concentrations tested, 2,3,7,8-TCDD had no effect on the transfectivity of QB RNA. Other compounds tested included the alkylating agents methyl, ethyl and isopropyl methane-sulfonate, and diethyl pyrocarbonate, all of which inactivated QB RNA under the same experimental conditions. The authors suggested that 2,3,7,8-TCDD inactivity in this assay indicated that 2,3,7,8-TCDD was an intercalating agent, and hence would require double stranded DNA in order to interact. The data presented in this study, however, were insufficient to support this conjecture.

*In vivo* binding of radiolabeled 2,3,7,8-TCDD to liver macromolecules was studied in Sprague-Dawley rats by Poland and Glover (1979). Both male and female animals were administered [1,6-$^3$H]2,3,7,8-TCDD i.p. at a dose of 7.5 μg/kg. This dose corresponded to a tritium level of 0.87 mCi/kg. The animals were killed 12, 48 and 168 hours after treatment, or 24 hours after treatment when the animals were pretreated with the enzyme inducers phenobarbital or unlabeled 2,3,7,8-TCDD. Following sacrifice, isolation of macromolecules, and removal of free labeled 2,3,7,8- TCDD, the amount of label bound to protein, RNA and DNA was determined. The greatest nonextractable binding of labeled 2,3,7,8-TCDD occurred to protein; however, the amount of label bound was small and only amounted to 0.03-0.1% of the total radioactivity administered. The total amount of label associated with RNA and DNA was, respectively, only 50 and 4 cpm above background. Time after exposure, sex or prior enzyme induction had no significant effect on 2,3,7,8-TCDD binding. As a result of the extremely low levels of radioactivity associated with RNA and DNA, it is uncertain whether 2,3,7,8-TCDD truly binds covalently to these macromolecules and, if so, whether there is any biological significance to this low level of apparent binding.

10.1.3. Cytogenetic Effects of 2,3,7,8-TCDD. The effects of 2,3,7,8-TCDD exposure on the extent of chromosomal aberrations in the bone marrow of male rats were reported in an abstract by Green and Moreland (1975). In the initial experiment, no increase in chromosomal aberration was observed after five daily gavage treatments at a 2,3,7,8-TCDD dose of 10 μg/kg. In the second portion of this study, rats were exposed by a single intraperitoneal injection of 2,3,7,8-TCDD at 5, 10 or 15 μg/kg or a single gavage treatment at 20 μg/kg. The animals at the two highest exposure levels were killed 24 hours post-treatment, while the remaining animals were killed 29 days post-treatment. Again, no increase in chromosomal aberrations was observed, except in the positive control group exposed to triethylenemelamine.

In a later report, a small but significant increase in chromosomal aberrations was observed in the bone marrow cells of male and female Osborne-Mendel rats (Green et al., 1977). Bone marrow cells for cytogenetic analysis were obtained from Osborne-Mendel rats used in a range-finding study preliminary to a chronic bioassay (Green et al., 1977). The animals in groups of 8 males and 8 females received twice weekly intubations of 2,3,7,8-TCDD at respective doses of 0.25, 1.0, 2.0 and 4.0, or 0.25, 0.5, 2.0 and 4.0 μg/kg for 13 weeks. Because it was not required for the range-finding study, a control group was not included. Bone marrow cells were analyzed for abnormalities and cells in mitosis in the animals that survived to the end of the study (4-8 animals/group). The only significant increases in chromosomal aberrations in comparison with the low dose group were in males at 2 and 4 μg/kg and females at 4 μg/kg. The greatest incidence observed was 4.65% of the cells with chromosomal breaks in the high-dose males; this was considered only weakly positive. The weak response, as well as the lack of data from control animals and the reported difficulty of obtaining cells from the high-dose animals as a result of 2,3,7,8-TCDD toxicity, makes the conclusion from this study that 2,3,7,8-TCDD produced chromosomal breaks tenuous.

A similar weak response was observed by Loprieno et al. (1982) in male and female CD-1 mice that received an intraperitoneal injection of 2,3,7,8-TCDD at a dose of 10 μg/kg. At 96 hours post-treatment, there was a significant ($p<0.01$) increase in bone marrow cells with gaps and chromatid aberrations. When chromosomal aberrations were analyzed at 24 hours post-treatment, there was no significant change in the incidence of cells with aberrant chromosomes. The study was continued with a more extensive experiment using CD-COBS female rats. The rats were treated weekly by gavage (vehicle acetone-corn oil 1:6) at doses of 0, 0.01, 0.10 or 1.00 μg/kg for 45 weeks. Analysis of bone marrow cells for chromosomal aberrations 24 hours after the last treatment failed to detect significant increases.

Czeizel and Kiraly (1976) reported an increased incidence ($p<0.001$) of chromatid-type and unstable chromosome aberrations in the peripheral lymphocytes of workers exposed to the herbicides 2,4,5-trichlorophenoxyethanol (2,4,5-TCPE) and Buminol. The 2,3,7,8-TCDD levels in the final product were <0.1 mg/kg; however, the exposure levels for individual workers were not available.

Mulcahy (1980) reported no increased incidences of chromosomal aberrations in the lymphocytes of 15 soldiers exposed to Agent Orange. The exposure was for 6-15 months and all subjects complained of symptoms, including skin eruptions, which they associated with Agent Orange. The analyses were performed with lymphocytes obtained ~10 years after the last exposure, and comparisons were made with eight subjects who had no history of exposure to 2,3,7,8-TCDD. Neither sister chromatid exchange nor structural aberrations including both gaps and breaks were increased. The authors noted that the long time between exposure and analysis may have accounted for the negative results.

In addition, Reggiani (1980) and Mottura et al. (1981) studied the 2,3,7,8-TCDD exposed inhabitants in Seveso. Reggiani (1980) examined 4 adults and 13 children (3-13 years) for chromosomal aberrations within 2 weeks of the accident. These 17 individuals were examined to support claims of and determine extent of injury. Although burn-like skin lesions in these 17 individuals indicated chemical exposure, no increase in chromosomal aberrations was detected. The methods of performing the analyses and the actual number of aberrations detected were not described. Similar negative results were reported in an abstract by Mottura et al. (1981). In this study, subjects were chosen from the area of heavy contamination following the accident (acute high level exposure), from the working population of the plant (chronic low level exposure) and a nonexposed control population. The number of subjects in each group was not provided. The specimens were examined by three independent laboratories and no laboratory reported an increase in chromosomal aberrations, although there was a significant difference in the reported scores between laboratories. There was no information in this abstract on the extent of individual exposure or the length of time that elapsed between the accident and obtaining samples for analyses of chromosomal aberrations.

Tenchini et al. (1979) also conducted a cytogenetic study of the exposed individuals at Seveso, Italy and of the aborted fetal tissue from exposed mothers. No significant chromosomal aberrations could be observed in the peripheral lymphocytes of the exposed idividuals. But aborted fetuses showed a nonsignificant increase in chromosomal abnormalities compared to the spontaneously aborted fetuses as observed in the general population. In a subsequent study, Tenchini et al. (1983) observed a significant increase in the frequencies of aberrant cells and in the average number of aberrations per damaged cell in fetal tissues from exposed pregnancies. This is a potentially interesting observation, but the study has the following pitfalls. First, the controls were nonconcurrent. This is a major problem in the interpretation of the results from pregnancies before and after exposure. Second, cells carrying the chromosomal aberrations described are not expected to survive more than one cell cycle, but in this study cells were examined that had undergone several cell divisions. This casts doubt on the validity of a positive result.

DiLernia et al. (1982) conducted additional studies on lymphocytes prepared in 1976 and 1979 from eight persons considered acutely exposed to 2,3,7,8-TCDD in the Seveso accident, eight ICMESA factory workers (considered chronically exposed), and 14 control subjects (eight had chromosome preparations made in 1976 and six in 1979). Cells were examined for average number of SAs (Satellite Associations; evidence for functional ribosomal genes), both on a cell basis and for the large acrocentric chromosomes (D group chromosomes). There was no change in the frequency of SAs on a per cell basis in any of the groups as compared to control values, nor in D group chromosomes from acutely exposed subjects examined immediately after the accident. There was, however, a decrease in the average frequency of SAs in group D chromosomes of acutely exposed subjects examined in 1977 and in ICMESA workers at both the 1976 and 1979 examinations. Although the biologic relevance of these observations has not yet been confirmed, DiLernia et al. (1982) observed a similar decrease in SAs after exposure of lymphocytes to x-irradiation. It was concluded that the decrease in SAs may have resulted from mutagenic damage to functional nucleolar organizing regions.

## 10.2. SUMMARY

A limited number of initial studies on the mutagenicity of 2,3,7,8-TCDD in bacteria reported positive results in _S_. _typhimurium_ strain TA1532 in the absence of a mammalian metabolic activation system (Hussain et al., 1972; Seiler, 1973). More recent attempts to repeat these results with strain TA1532 or related strains have failed (Geiger and Neal, 1981; Nebert et al., 1976; Gilbert et al., 1980; McCann, 1978). These authors have also reported no increase in mutation rate when 2,3,7,8-TCDD was tested in the presence of a mammalian metabolic activation system. In other _in vitro_ assays, 2,3,7,8-TCDD has produced a positive response in reversion to streptomycin independence in _E_. _coli_ Sd-4 cells and questionable positive response with prophage induction in _E_. _coli_ K-39 cells (Hussain et al., 1972). Also, 2,3,7,8-TCDD has been reported to be mutagenic in the yeast _S_. _cerevisiae_ in both the _in vitro_ assay with S-10 and the host-mediated assay (Bronzetti et al., 1983). Rogers et al. (1982) also reported positive mutagenicity results in the mouse lymphoma assay system. In the _E_. _coli_ studies, the poor survival of the cells or the interference of the vehicle solvent, DMSO, with the assay makes the evaluation of the studies difficult. With the data available, it is not possible to resolve the conflicting reports on the mutagenic potential of 2,3,7,8-TCDD.

Overall, the data indicate little potential for the interaction of 2,3,7,8-TCDD with nucleic acids or the ability of 2,3,7,8-TCDD to produce chromosomal aberrations. Kondorosi et al. (1973) demonstrated that 2,3,7,8-TCDD did not react with RNA _in vitro_ in the absence of a metabolic activation system. _In vivo_ studies using radiolabeled 2,3,7,8-TCDD indicated some association of nonextractable label with RNA and DNA (Poland and Glover, 1979); however, the level of bound label was very low. Similar marginal data were available on the clastogenic effect of 2,3,7,8-TCDD. Although two in vivo studies in rats (Green and Moreland, 1975; Loprieno et al., 1982) failed to demonstrate treatment-related chromosomal aberration, a second study by the same authors (Green et al., 1977) using a longer exposure period reported a small increase in the number of aberrations. A similar small increase was observed by Loprieno et al. (1982) following a single intraperitoneal injection of 2,3,7,8-TCDD in mice. In humans exposed to 2,3,7,8-TCDD during the manufacture of 2,4,5-TCPE and Buminol, Czeizel and Kiraly (1976) reported an increase in the number of chromosomal aberrations; however, no increase was detected in individuals exposed to 2,3,7,8-TCDD following an industrial accident in Seveso, Italy (Reggiani, 1980; Mottura et al., 1981; Tenchini et al., 1979). In contrast, Tenchini et al. (1983) reported positive results in a Seveso study, but this study has problems. The studies of the clastogenic effect of 2,3,7,8-TCDD were presented with little or no experimental detail to assist in evaluating the merits of the reports. The data available are too limited to indicate whether 2,3,7,8-TCDD can interact with nucleic acids or produce chromosomal aberrations.

The differences among the results reported could be due to several factors, such as treatment protocols, solubility problems, purity of the samples tested and the high toxicity of 2,3,7,8-TCDD. This chemical may be a weak mutagen, but because it is very toxic, the dose range for detecting a positive genetic effect may be very narrow. Therefore, additional experimentation is necessary before any conclusive determination can be made. Suggested further testing includes the ability of 2,3,7,8-TCDD to induce forward mutations in mammalian cells in culture, additional yeast and bacterial studies and the sex-linked recessive lethal test in *Drosophila*.

Pertinent information regarding the mutagenicity of PeCDDs and HxCDDs were not located in the available literature.

# 11. CARCINOGENICITY

The purpose of this section is to provide an evaluation of the likelihood that 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), and a mixture of 1,2,3,7,8,9- and 1,2,3,6,7,8-hexachlorodibenzo-p-dioxin (HxCDD), are human carcinogens and, on the assumption that they are human carcinogens, to provide a basis for estimating their public health impact, including a potency evaluation, in relation to other carcinogens. The evaluation of carcinogenicity depends heavily on animal bioassays and epidemiologic evidence. However, information on mutagenicity and metabolism, particularly in relation to interaction with DNA, as well as to pharmacokinetic behavior, has an important bearing on both the qualitative and quantitative assessment of carcinogenicity. The available information on these subjects is reviewed in other sections of this document. This chapter presents an evaluation of the animal bioassays, the human epidemiologic evidence, the quantitative aspects of assessment, and finally, a summary and conclusions section dealing with all of the relevant aspects of carcinogenicity.

## 11.1. ANIMAL STUDIES

11.1.1. Studies Using 2,3,7,8-TCDD. The polychlorinated dibenzo-p-dioxins (PCDDS), 2,3,7,8-TCDD and a mixture of 1,2,3,7,8,9- and 1,2,3,6,7,8-HxCDD, have been tested for carcinogenicity in rats and mice by administering the compound in the diet and by gavage. Also, the tumor incidence in native mice inhabiting an area with heavy exposure to the herbicide Agent Orange has been assessed and compared with mice from an uncontaminated habitat. The results of these bioassays are discussed in this section. Along with studies using the oral route, both 2,3,7,8-TCDD and a mixture of 1,2,3,7,8,9- and 1,2,3,6,7,8-HxCDD have been tested for tumorigenicity by dermal application. Using the skin two-stage tumorigenicity model, 2,3,7,8-TCDD has been tested for promoting and initiating activity as well as anticarcinogenic activity. Other model systems have been used to a more limited extent in studies of the effect of 2,3,7,8-TCDD on the carcinogenic potential of chemical carcinogens.

11.1.1.1. VAN MILLER ET AL. (ORAL) RAT STUDY (1977a,b) -- In a limited study, Van Miller et al. (1977a,b) maintained small groups of male Sprague-Dawley rats on diets containing 2,3,7,8-TCDD. The animals, in groups of 10, were fed diets containing 0.0, 0.001, 0.005, 0.05, 0.5, 1.0, 5.0, 50, 500 or 1000 ppb of 2,3,7,8-TCDD for 78 weeks. As determined from the food consumption of two animals from each group, these exposure levels corresponded to doses of 0.0, 0.0003, 0.001, 0.01, 0.1, 0.4, 2.0, 2.4, 240 and 500 μg/kg/week, respectively. At week 65 of treatment, all surviving animals were examined by laparotomy, and biopsy samples were obtained from any gross tumors. Following termination of treatment, the animals were observed for an additional 17 weeks before sacrificing all surviving animals. Necropsy was performed on animals killed when moribund, found dead or killed at termination of the study, and the animals were examined for both gross and microscopic lesions. Intake and mortality are shown in Table 11-1.

All animals in groups maintained on diets containing 1-1000 ppb of 2,3,7,8-TCDD were dead by week 90 of treatment; the first deaths in groups at the 1000 and 1 ppb levels were observed at 2 weeks and 31 weeks of treatment, respectively. Animals exposed to 0.001-0.5 ppb of 2,3,7,8-TCDD had similar food consumption and survival as control animals; however, all treated animals had histopathologic degenerative changes in the kidneys.

## TABLE 11-1

## 2,3,7,8-TCDD Intake and Mortality in Male Sprague-Dawley Rats[a]

| Dose[b] (ppb) | Weekly Dose/Rat (μg/kg bw) | Week of First Death | Number of Rats Dead at 95th Week |
|---|---|---|---|
| 0.0 | -- | 68 | 6/10 (60%) |
| 0.001 | 0.0003 | 86 | 2/10 (20%) |
| 0.005 | 0.001 | 33 | 4/10 (40%) |
| 0.05 | 0.01 | 69 | 4/10 (40%) |
| 0.5 | 0.1 | 17 | 5/10 (50%) |
| 1 | 0.4 | 31 | 10/10 (100%) |
| 5 | 2.0 | 31 | 10/10 (100%) |

[a] Source: Van Miller et al., 1977a,b

[b] Rats at 50, 500 and 1000 ppb dose levels were all dead within 4 weeks.

Complete necropsies were done and samples of tissues were taken for microscopic examination from the control groups and each treatment group (Laboratory audit* and personal communication with author).

Special staining methods were used as an aid in the diagnosis of neoplasms. Various benign and malignant tumors were found in each treatment group. No tumors were observed in the controls (Table 11-2).

Statistically significant increases of squamous cell tumors of the lungs and neoplastic nodules of the liver were observed in rats ingesting 5 ppb TCDD (Table 11-3). In addition, two animals in the 5 ppb dose group and one animal in the 1 ppb dose group had liver cholangiocarcinomas, which are rare in Sprague-Dawley rats. These results provide evidence of a carcinogenic effect.

The observation of no tumors of any kind in the controls is unusual for Sprague-Dawley rats. In addition, the reporting of the study was not extensive. These factors may tend to lessen the reliance that can be placed on the positive results of this study. However, this study is suggestive of a carcinogenic response upon exposure to TCDD in rats.

11.1.1.2. KOCIBA ET AL. (ORAL) RAT STUDY (1978a) -- Although this study was published as Kociba et al., 1978a, a fuller version was submitted in an unpublished report (Kociba et al., 1977).

In this study, groups of 50 Sprague-Dawley rats (Spartan substrain) of each sex were maintained for up to 2 years on diets providing 0.1, 0.01 or 0.001 μg/kg/day 2,3,7,8-TCDD. Vehicle control groups consisted of 86 animals of each sex. The test was appropriately conducted with the

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*The audit of this study brought out the fact that it was intended to be only a range-finding study. Therefore, only small numbers of animals were used. This may have made the study relatively insensitive for detecting carcinogenic effects at doses <1 ppb.

## TABLE 11-2

### Benign and Malignant Tumors in Rats Ingesting 2,3,7,8-TCDD[a]

| Dose[b] | Benign | Malignant | Number of Tumors | Number of Rats With Tumors |
|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0/10 (0%)[c] |
| 1 ppt | 0 | 0 | 0 | 0/10 (0%) |
| 5 ppt | 1 | 5 | 6[d] | 5/10 (50%)[e] |
| 50 ppt | 2 | 1 | 3[f] | 3/10 (30%) |
| 500 ppt | 2 | 2 | 4[g] | 4/10 (40%)[h] |
| 1 ppb | 0 | 4 | 5[i] | 4/10 (40%) |
| 5 ppb | 8 | 2 | 10[j] | 7/10 (70%) |

[a] Source: Van Miller et al., 1977a,b

[b] Rats at dose levels of 50, 500 and 1000 ppb were all dead within 4 weeks.

[c] 40 male rats used as controls for another study, received at the same time and kept under identical conditions, did not have neoplasms when killed at 18 months.

[d] 1 rat had ear duct carcinoma and lymphocytic leukemia
1 adenocarcinoma (kidney)
1 malignant histiocytoma (retroperitoneal)
1 angiosarcoma (skin)
1 Leydig cell adenoma (testis)

[e] 3 rats died with aplastic anemia

[f] 1 fibrosarcoma (muscle)
1 squamous cell tumor (skin)
1 astrocytoma (brain)

[g] 1 fibroma (striated muscle)
1 carcinoma (skin)
1 adenocarcinoma (kidney)
1 sclerosing seminoma (testis)

[h] 1 rat had a severe liver infarction

[i] 1 rat cholangiocarcinoma and malignant histiocytomas (retroperitoneal)
1 angiosarcoma (skin)
1 glioblastoma (brain)
1 malignant histiocytoma (retroperitoneal)

[j] 1 rat had squamous cell tumor (lung) and neoplastic nodule (liver)
2 cholangiocarcinomas and neoplastic nodules (liver)
3 squamous cell tumors (lung)
1 neoplastic nodule (liver)

## TABLE 11-3

### Liver Tumors in Rats Ingesting 2,3,7,8-TCDD[a]

| Dose (ppb) | Neoplastic Nodules | Cholangiocarcinomas | Squamous Cell Tumors of the Lungs |
|---|---|---|---|
| 0 | 0/10 (0%) | 0/10 (0%) | 0/10 |
| 1 | 0/10 (0%) | 1/10 (10%) | 0/10 |
| 5 | 4/10 (40%)<br>p=0.043[c] | 2/10 (20%)[b] | 4/10 (40%)<br>p=0.043[c] |

[a] Source: Van Miller et al., 1977a,b

[b] The two animals had both neoplastic nodules of the liver and cholangiocarcinomas.

[c] p-values calculated using the Fisher Exact Test.

high-dose group given a dose which induced signs of tissue toxicity, reduced weight increments in both sexes, and shortened lifespans in female rats. Clinical tests performed at intervals during the study monitored organ specific toxicity, particularly of the liver. Pathologic examinations included histopathologic evaluation of all major tissues in both the high-dose and control animals, but only of selected tissues identified as possible target organs and suspect tumors in lower-dose groups. This approach is suitable for the identification of a carcinogenic effect, but does not determine actual tumor incidences in all groups except in those organs identified as target organs. It, therefore, is adequate to define dose-response relationships only in these target organs. Tissues examined from most animals in all dose groups included liver, lungs, kidneys, urinary bladder, tongue, brain, testes/ovaries and prostate/uterus. For these tissues, a quantitative analysis can be performed using the actual number of tissues examined histopathologically for animals at risk. For other tissues (excluding skin, mammary glands and nasal turbinates/hard palate), actual tumor incidence cannot be evaluated for the two lower doses. For skin, mammary glands and nasal turbinates/hard palate, the number of animals necropsied is the appropriate denominator to determine incidence, because detection of these tumors is based on observation of the tumor at necropsy.

A laboratory audit of this study by H. Spencer and W.S. Woodrow, Hazard Evaluation Division, Office of Pesticide Programs, U.S. EPA, did not reveal significant new information. Reviewers concluded that the study was properly conducted, adhering to the accepted procedures (Spencer and Woodrow, 1979).

Based on data reported for food consumption, body weight and dietary level of TCDD, the daily doses were reasonably constant for most of the study, although somewhat below the value expected in most groups during the third month.

High early mortality was observed in all groups in this study but was only statistically significant in the high-dose group. The survival curves show progressive mortality beginning as early as the 12th month and leading to 50% mortality by 21 months.* The effects of this early mortality are a reduction in expected tumor incidence because of a truncated latency period, and a reduction in sensitivity of the study because of a reduction in number of animals at risk during the time of expected tumor manifestation. Cumulative mortality and interval mortality rates are given in Tables A-1 to A-4 of Appendix A (Clement Associates, 1979).

The results of this study provide substantial evidence that 2,3,7,8-TCDD is carcinogenic in rats. 2,3,7,8-TCDD induced a highly statistically significant increase of both hepatocellular carcinomas and hepatocellular neoplastic nodules in female rats at doses of 0.1 and 0.01 μg/kg/day (2200 and 210 ppt in the diet, respectively). The increase of hepatocellular carcinomas alone, in the high-dose females, was also highly significant. In addition, at the highest dose level, 2,3,7,8-TCDD induced a statistically significant increase in stratified squamous cell carcinomas of the hard palate and/or nasal turbinates in both males and females, squamous cell carcinomas of the tongue in males, and highly significant keratinizing squamous cell carcinomas of the lungs in females (Tables 11-4, 11-5 and 11-6).

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*In the 0.001 group of males, 44% of the animals had died by 18 months. The mortality patterns were analyzed by the Whitney-Wilcoxon test and the Kolmogorov-Simonov test. These tests showed that mortality was significantly higher in the high-dose females than in controls, and while indications of increased mortality were found in other groups, they were not part of a consistent pattern.

## TABLE 11-4

### Hepatocellular Carcinomas and Hepatocellular Hyperplastic Nodules in Female Sprague-Dawley Rats Maintained on Diets Containing 2,3,7,8-TCDD[a]

| Dose Level (μg/kg/day) | Hepatocellular Hyperplastic Nodules | Hepatocellular Carcinomas[b] | Total Number With Both Types of Tumors[b] |
|---|---|---|---|
| 0 | 8/86 (9%) | 1/86 (1%) | 9/86 (10%) |
| 0.001<br>(22 ppt) | 3/50 (6%) | 0/50 (0%) | 3/50 (6%) |
| 0.01<br>(210 ppt) | 18/50 (36%) | 2/50 (4%) | 18/50 (36%)[c]<br>($p=4.36 \times 10^{-4}$) |
| 0.1<br>(2200 ppt) | 23/49 (48%) | 11/49 (22%)<br>($p=5.6 \times 10^{-5}$) | 34/50 (71%)<br>($p=4.56 \times 10^{-13}$) |

[a] Source: Kociba et al., 1977

[b] p-values calculated using the Fisher Exact Test (one-tailed).

[c] Two rats had both hepatocellular carcinomas and hyperplastic nodules.

## TABLE 11-5

### Tumor Incidence in Female Rats Fed Diets Containing 2,3,7,8-TCDD[a]

| Dose Level (μg/kg/day) | Stratified Squamous Cell Carcinomas of Hard Palate or Nasal Turbinates | Keratinizing Squamous Cell Carcinomas of Lungs |
|---|---|---|
| 0 | 1/54 (2%) | 0/86 (0%) |
| 0.001<br>(22 ppt) | 0/30 (0%) | 0/50 (0%) |
| 0.01<br>(210 ppt) | 1/27 (4%) | 0/50 (0%) |
| 0.1<br>(2200 ppt) | 5/24 (21%)<br>(p=0.01)[b] | 7/49 (14%)<br>(p=0.0006)[b] |

[a] Source: Kociba et al., 1977

[b] p-values calculated using the Fisher Exact Test (one-tailed).

### TABLE 11-6

### Tumor Incidence in Male Rats Fed Diets Containing 2,3,7,8-TCDD[a]

| Dose Level (μg/kg/day) | Stratified Squamous Cell Carcinomas of the Tongue | Hard Palate/Nasal Turbinates Stratified Squamous Cell Carcinoma[b] |
|---|---|---|
| 0 | 0/76 (0%) | 0/51 (0%) |
| 0.001<br>(22 ppt) | 1/49 (2%) NS | 1/34 (3%) NS |
| 0.01<br>(210 ppt) | 1/50 (2%) NS | 0/27 (0%) NS |
| 0.1<br>(2200 ppt) | 3/42 (7%) ($p=4.3 \times 10^{-2}$)[c] | 4/30 (13%) ($p=0.016$)[c] |

[a] Source: Kociba et al., 1977

[b] Includes examinations from both original and updated report (5/20/79).

[c] p-values calculated using the Fisher Exact Test.

NS = Not significant at $p=0.05$.

Dr. Robert Squire, pathologist at the Johns Hopkins University Medical School and consultant to the CAG, evaluated the histopathologic slides from Dow Chemical Company's 2-year rat feeding studies on 2,3,7,8-TCDD by Kociba et al. (1978a). Dr. Squire and his associates examined all liver, lungs, tongues, hard palates and nasal turbinates available from the 2,3,7,8-TCDD study. Their histopathological findings, as well as Dr. Kociba's histopathological evaluations, are summarized in Tables 11-7 and 11-8 and Appendix B. Although there are some differences between the diagnoses of Drs. Kociba and Squire, the conclusions about the target organ for cancer induction and the dose levels at which induction occurred are the same.

11.1.1.3. **NATIONAL TOXICOLOGY BIOASSAY PROGRAM (ORAL) RAT STUDY (1980a,b)** -- A cancer bioassay for the possible carcinogenicity of 2,3,7,8-TCDD was tested by the Illinois Institute of Technology in rats and mice under a contract sponsored by the National Cancer Institute (NCI).

In the rat study, 50 Osborne-Mendel rats of each sex were administered 2,3,7,8-TCDD* suspended in a vehicle of 9:1 corn oil-acetone by gavage 2 days/week for 104 weeks at doses of 0.01, 0.05 or 0.5 μg/kg/week. Seventy-five rats of each sex served as vehicle controls. One untreated control group containing 25 rats of each sex was present in the 2,3,7,8-TCDD treatment room and one untreated control group containing 25 rats of each sex was present in the vehicle control room. All surviving rats were killed at 105-107 weeks.

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*Purity of 2,3,7,8-TCDD was found to be 99.4%; two impurities tentatively identified as a trichlorodibenzo-p-dioxin and a pentachlorodibenzo-p-dioxin. The presence of 0.1-0.2% hexachlorodibenzo-p-dioxin was also detected by gas chromatography and mass spectrometry.

TABLE 11-7

Dow 2,3,7,8-TCDD Oral Rat Study by Dr. Kociba, With Dr. Squire's Review (8/15/80)
Sprague-Dawley Female Rats - Spartan Substrain (2 years)[a,b]

| Tissues and Diagnoses | Dose Levels (μg/kg/day) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 (control) | | 0.001 | | 0.01 | | 0.1 | |
| | S | K | S | K | S | K | S | K |
| Lung<br>Squamous cell carcinomas | 0/86 | 0/86 | 0/50 | 0/50 | 0/49 | 0/49 | 8/47 (17%)<br>($p=1.61 \times 10^{-4}$) | 7/49 (14%)<br>($p=6.21 \times 10^{-4}$) |
| Nasal turbinate/hard palate squamous cell carcinomas | 0/54 | 1/54 | 0/30 | 0/30 | 1/27 | 1/27 | 5/22 (23%)<br>($p=1.43 \times 10^{-3}$) | 5/24 (21%)<br>($p=9.46 \times 10^{-3}$) |
| Liver<br>Neoplastic nodules/ hepatocellular carcinomas | 16/86 | 9/86 | 8/50 | 3/50<br>($p=4.37 \times 10^{-4}$) | 27/50<br>($p=2.42 \times 10^{-5}$) | 18/50<br>($p=4.37 \times 10^{-4}$) | 33/47 (70%)<br>($p=4.92 \times 10^{-8}$) | 34/48 (71%)<br>($p=9.53 \times 10^{-13}$) |
| Total combined (each animal had at least one tumor above) | 16/86<br>19% | 9/86<br>10% | 8/50<br>16% | 3/50<br>6%<br>($p=4.37 \times 10^{-4}$) | 27/50<br>54%<br>($p=2.42 \times 10^{-5}$) | 18/50<br>34%<br>($p=4.37 \times 10^{-4}$) | 34/47<br>72%<br>($p=1.20 \times 10^{-9}$) | 34/49<br>69%<br>($p=2.13 \times 10^{-12}$) |

[a] Source: Kociba et al., 1977; Squire, 1980

[b] p-values calculated using the Fisher Exact Test.

S = Dr. Squire's histopathologic analysis; K = Dr. Kociba's histopathologic analysis

## TABLE 11-8

### Dow 2,3,7,8-TCDD Oral Rat Study by Dr. Kociba, With Dr. Squire's Review (8/15/80)
### Sprague-Dawley Male Rats - Spartan Substrain (2 years)*

| Tissues and Diagnoses | Dose Levels (μg/kg/day) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 (control) | | 0.001 | | 0.01 | | 0.1 | |
| | S | K | S | K | S | K | S | K |
| Nasal turbinate/hard palate squamous cell carcinomas | 0/55 | 0/51 | 1/34 | 1/34 | 0/26 | 0/27 | 6/30 (20%)<br>(p=1.36 x $10^{-3}$) | 4/30 (13%)<br>(p=1.6 x $10^{-2}$) |
| Tongue squamous cell carcinomas | 0/77 | 0/76 | 2/44 | 1/49 | 1/49 | 1/49 | 3/44 (7%)<br>(p=4.60 x $10^{-2}$) | 3/42 (7%)<br>(p=4.34 x $10^{-2}$) |
| Total - 1 or 2 above (each rat had at least one tumor above) | 0/77 | | 2/44<br>5% | | 1/49<br>2% | | 9/44<br>20%<br>(p=6.28 x $10^{-5}$) | |

*p-values calculated using the Fisher Exact Test.

S = Dr. Squire's histopathologic analysis

K = Dr. Kociba's histopathologic analysis

In rats, a dose-related depression in mean body weight gain became evident in the males after week 55 of the bioassay and in the females after week 45.

The results of histopathologic diagnosis of primary tumors caused by the oral administration of 2,3,7,8-TCDD are presented in Table 11-9. In male rats an increased incidence of follicular-cell adenomas or carcinomas of the thyroid was dose-related and was statistically significantly higher in the low-, mid- and high-dose groups than in the vehicle controls. In addition, a statistically significant increase in subcutaneous tissue fibromas was found in males of the high-dose group.

In female rats, a statistically significant increase of each of the following tumors was found in the high-dose group: hepatocellular carcinomas and neoplastic nodules (p=0.001), subcutaneous tissue fibrosarcomas (p=0.023) and adrenal cortical adenomas (p=0.039), as shown in Table 11-10.

These results confirm the carcinogenic effect observed in the Kociba et al. (1978a) study using Sprague-Dawley (Spartan substrain) rats.

11.1.1.4. TOTH ET AL. (ORAL) MOUSE STUDY (1979) -- This study investigated the carcinogenicity of 2,3,7,8-TCDD in Swiss mice. Ten-week-old outbred Swiss/H/Riop mice were used. 2,3,7,8-TCDD was administered in a sunflower oil vehicle by gavage to groups of 45 male mice once a week at doses of 7.0, 0.7 and 0.007 μg/kg bw for a year (groups 9, 10, 11, respectively, in Table 11-11). Matched male vehicle controls were administered sunflower oil once a week. Matched controls to a companion study investigating the carcinogenicity of (2,3,5-trichlorophenoxy)ethanol (TCPE) contaminated with low levels of 2,3,7,8-TCDD, were administered carboxymethyl cellulose (the vehicle used in that study) once a week. Two untreated controls were also maintained.

## TABLE 11-9

### Incidence of Primary Tumors in Male Rats Administered 2,3,7,8-TCDD by Gavage[a]

| Type of Tumor | Vehicle Control | µg/kg/week | | |
|---|---|---|---|---|
| | | Low Dose[b] 0.01 | Mid Dose[b] 0.05 | High Dose[b] 0.5 |
| Subcutaneous tissue Fibroma | 3/75 (4%) | 1/50 (2%) | 3/50 (6%) | 7/50 (14%)<br>p=0.048 |
| Liver Neoplastic nodule or hepatocellular carcinoma | 0/74 (0%) | 0/50 (0%) | 0/50 (0%) | 3/50 (6%) |
| Adrenal Cortical adenoma | 6/72 (8%) | 9/50 (18%) | 12/49 (24%) | 9/49 (18%) |
| Thyroid Follicular cell adenoma | 1/69 (1%) | 5/48 (10%)<br>p=0.042 | 6/50 (16%)<br>p=0.021 | 10/50 (20%)<br>p=0.001 |
| Thyroid Follicular cell adenoma or carcinoma | 1/69 (1%) | 5/48 (10%)<br>p=0.042 | 8/50 (16%)<br>p=0.004 | 11/50 (22%)<br>p<0.001 |

[a] Source: NTP, 1980a

[b] p-values calculated using the Fisher Exact Test.

## TABLE 11-10

### Incidence of Primary Tumors in Female Rats Administered 2,3,7,8-TCDD by Gavage[a]

| | µg/kg/week | | | |
|---|---|---|---|---|
| Type of Tumor | Vehicle Control | Low Dose[b] 0.01 | Mid Dose 0.05 | High Dose[b] 0.5 |
| Subcutaneous tissue<br>Fibrosarcoma | 0/75 (0%) | 2/50 (4%) | 3/50 (6%) | 4/49 (8%)<br>p=0.023 |
| Liver<br>Neoplastic nodule | 5/74 (7%) | 1/49 (2%) | 3/50 (6%) | 12/49 (24%)<br>p=0.006 |
| Liver<br>Neoplastic nodule or hepatocellular carcinoma | 5/75 (7%) | 1/49 (2%) | 3/50 (6%) | 14/49 (29%)<br>p=0.001 |
| Pituitary<br>Adenoma | 1/66 (2%) | 5/47 (11%)<br>p=0.044 | 2/44 (5%) | 3/43 (7%) |
| Adrenal<br>Cortical adenoma | 11/73 (15%) | 8/49 (16%) | 4/49 (8%) | 14/46 (30%)<br>p=0.039 |

[a] Source: NTP, 1980a

[b] p-values calculated using the Fisher Exact Test.

TABLE 11-11

Cumulative Data on Tumor Incidence[a]

| Group | TCPE[b] (mg/kg) | Treatment<br>TCDD (μg/kg) | <br>Vehicle[c] (mg/kg) | Sex | Effective Number of Mice | Number of Tumor Bearing Mice | Number of Animals with Tumors of:<br>Liver (%) | <br>Lung | <br>Lymphomas | <br>Other Organs | Average Lifespan |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 67.0 | 0.112 (1.6 ppm) | 50 | M | 88 | 69 | 42[d] (18) | 50 | 7 | 16 | 595 |
| | | | | F | 83 | 61 | 7 (8) | 52 | 15 | 25 | 652 |
| 2 | 70.0 | 0.007 (0.1 ppm) | 50 | M | 98 | 78 | 57[e] (58) | 18 | 11 | 16 | 571 |
| | | | | F | 96 | 59 | 9 (9) | 39 | 15 | 23 | 582 |
| 3 | | control | | M | 93 | 63 | 24 (26) | 44 | 8 | 17 | 577 |
| | | | | F | 84 | 57 | 4 (5) | 41 | 23 | 13 | 639 |
| 4 | 7.0 | 0.07 (10 ppm) | 50 | M | 93 | 79 | 25 (27) | 38 | 18 | 22 | 641 |
| | | | | F | 96 | 60 | 10 (10) | 38 | 19 | 19 | 589 |
| 5 | 7.0 | 0.0007 (0.1 ppm) | 50 | M | 94 | 77 | 23 (24) | 50 | 23 | 17 | 660 |
| | | | | F | 93 | 71 | 8 (9) | 42 | 36 | 21 | 590 |
| 6 | 0.7 | 0.00007 (0.1 ppm) | 50 | M | 97 | 78 | 24 (25) | 51 | 20 | 17 | 643 |
| | | | | F | 94 | 64 | 5 (5) | 38 | 22 | 21 | 566 |
| 7 | -- | -- | 50 | M | 96 | 74 | 32 (33) | 44 | 14 | 22 | 615 |
| | | | | F | 84 | 55 | 4 (5) | 38 | 18 | 17 | 565 |
| 8 | | control | | M | 96 | 78 | 32 (33) | 38 | 22 | 15 | 651 |
| | | | | F | 91 | 57 | 4 (4) | 31 | 24 | 19 | 549 |
| 9 | | 7.0 | 10 | M | 43 | 27 | 13 (30) | 11 | 6 | 7 | 424 |
| 10 | | 0.7 | 10 | M | 44 | 36 | 21 (48) | 18 | 12 | 4 | 633 |
| 11 | | 0.007 | 10 | M | 44 | 39 | 13 (29) | 27 | 10 | 6 | 649 |
| 12 | -- | -- | 10 | M | 38 | 27 | 7 (18) | 15 | 6 | 7 | 588 |

[a] Source: Toth et al., 1979

[b] TCPE = Trichlorophenoxy ethanol

[c] Carboxymethyl cellulose in groups 1-8, sunflower oil in groups 9-12.

[d] $p<1\%$

[e] $p<0.1\%$

This study appears to have been generally well conducted. However, the administration of 2,3,7,8-TCDD over a period of only 1 year, which is far short of the life expectancy of the mice used, made the study relatively insensitive. Animals were followed for their entire lifetimes. Autopsies were performed after spontaneous death or when the mice were moribund, and all organs were examined histologically. Sections were stained with hematoxylin and eosin for light microscopy. Pathological findings were evaluated and analyzed statistically. The findings of the 2,3,7,8-TCDD study and the comparison study on TCPE are given in Table 11-11.

Analysis of the results of this study focused on the incidence of liver tumors in the groups treated with 2,3,7,8-TCDD and the incidence of these tumors in the matched controls (group 12) and in the males in the three other control groups. Males in groups 3 and 8, the two untreated control groups, had 26% and 33% liver tumors, respectively ($p<0.20$). The carboxymethyl cellulose male controls (group 7) had 33% (32/96) liver tumors. No significant differences in liver tumors were observed when males in all four control groups were compared with each other ($p<0.05$). Nevertheless, there was evidence that the incidence of liver tumors in the control groups was associated with the average lifespan in the respective groups. The two groups that had <600 days average survival (groups 3 and 12) had the fewest liver tumors (26 and 18%, respectively). On the other hand, the two groups that had an average survival of >600 days (groups 7 and 8), had 33% liver tumors each. The test for linear trend (tumors vs. days of average survival) was not quite significant ($p=0.065$).

Among the three treatment groups (groups 9, 10 and 11), the middle dose (0.7 μg/kg) showed the highest incidence of liver tumors (21/44 = 48%).

This incidence was significantly higher than the incidence of liver tumors in either the sunflower oil controls ($p<0.01$) or the pooled controls (all four control groups combined) ($p<0.025$).

The highest-dose group (7.0 μg/kg) had an increased incidence of liver tumors compared with the matched sunflower oil controls (13/43 = 30%), but this increase was not statistically significant ($p=0.11$). The incidence of liver tumors in the high-dose group was comparable with that of the pooled controls. The highest-dose group, however, had a much reduced average survival in comparison with any of the control groups (only 424 days compared with 577, 588, 615 and 651 days in the four control groups). This poor survival may have accounted for the lack of a statistically significant increase in liver tumors in the high-dose group. Furthermore, if time-to-tumor data had been available, it is likely that the high-dose group would have shown a significant decrease in time-to-tumor compared with the controls. Therefore, the increase in liver tumors that was observed in the high-dose group in comparison with the matched control group, although not statistically significant, is considered to be consistent with an oncogenic effect.

In conclusion, the results of this study provide suggestive evidence of an oncogenic effect.

11.1.1.5. NATIONAL TOXICOLOGY BIOASSAY PROGRAM (ORAL) MOUSE STUDY (1980a,b) -- A cancer bioassay for the possible carcinogenicity of 2,3,7,8-TCDD was tested by the Illinois Institute of Technology in mice under a contract sponsored by the NCI.

In the mouse study, groups of 50 B6C3F1 mice of each sex were administered 2,3,7,8-TCDD suspended in a vehicle of 9:1 corn oil-acetone 2 days/week for 104 weeks at doses of 0.01, 0.05 and 0.5 μg/kg/week for male mice and 0.04, 0.2 and 2.0 μg/kg/week for female mice. Seventy-five mice of each sex were used as vehicle controls. One untreated control group of 25 mice of each sex was present in the 2,3,7,8-TCDD treatment room. One untreated control group of 25 mice of each sex was present in the vehicle control room. In mice, the mean body weight gain in the treated groups was comparable with that of the vehicle control groups. However, the mean body weight of the treated mice was lower when it was compared with untreated controls.

The results of the histopathologic diagnosis of primary tumors are presented in Table 11-12. The results indicate that, in male mice, 2,3,7,8-TCDD induced a statistically significant incidence of hepatocellular carcinomas (p=0.002) and both hepatocellular carcinomas and neoplastic nodules combined (p=<0.001) in male mice of the high-dose group.

In female mice, 2,3,7,8-TCDD induced statistically significant increases of hepatocellular carcinomas (p=0.014) and both hepatocellular adenomas and carcinomas (p=0.002) in the high-dose group. In addition, a statistically significant increase in tumor incidences of fibrosarcoma, histiocytic lymphoma, thyroid follicular-cell adenoma and cortical adenoma or carcinoma were also observed in the high-dose group (Table 11-13).

The incidence of liver tumors observed in this study confirms the earlier observations of an increase in liver tumors in the male mouse study performed by Toth et al. (1979).

#### 11.1.1.6. OTHER RELATED STUDIES --

11.1.1.6.1. Pitot et al. Promotion Study in Rats (1980) -- Pitot et al. (1980) investigated a two-stage model of hepatocarcinogenesis. Twenty-four hours after a partial hepatectomy (to enhance cell proliferation), female Sprague-Dawley rats were divided into seven groups (Table 11-14).

## TABLE 11-12

### Incidence of Primary Tumors in Male Mice Administered 2,3,7,8-TCDD by Gavage[a]

| Type of Tumor | Vehicle Control | µg/kg/week<br>Low Dose<br>0.01 | <br>Mid Dose<br>0.05 | <br>High Dose[b]<br>0.5 |
|---|---|---|---|---|
| Liver Hepatocellular adenoma | 7/73 (10%) | 3/49 (6%) | 5/49 (10%) | 10/50 (20%) |
| Liver Hepatocellular carcinoma | 8/73 (11%) | 9/49 (18%) | 8/49 (16%) | 17/50 (34%)<br>p=0.002 |
| Liver Hepatocellular adenoma and carcinoma | 15/73 (21%) | 12/49 (24%) | 13/49 (27%) | 27/50 (54%)<br>p=<0.001 |

[a] Source: NTP, 1980a

[b] p-values calculated using the Fisher Exact Test.

## TABLE 11-13

## Incidence of Primary Tumors in Female Mice Administered 2,3,7,8-TCDD by Gavage[a]

| Type of Tumor | Vehicle Control | µg/kg/week<br>Low Dose 0.04 | <br>Mid Dose 0.2 | <br>High Dose[b] 2.0 |
|---|---|---|---|---|
| Subcutaneous tissue Fibrosarcoma | 1/74 (1%) | 1/50 (2%) | 1/48 (2%) | 5/47 (11%)<br>p=0.032 |
| Hematopoietic system Histiocytic lymphoma | 9/74 (12%) | 4/50 (8%) | 4/48 (17%) | 14/47 (30%)<br>p=0.016 |
| Hematopoietic system All lymphoma | 18/74 (24%) | 11/50 (22%) | 13/48 (27%) | 20/47 (43%)<br>p=0.029 |
| Hematopoietic system Lymphoma or leukemia | 18/74 (24%) | 12/50 (24%) | 13/48 (27%) | 20/47 (43%)<br>p=0.029 |
| Liver Hepatocellular carcinoma | 1/73 (1%) | 2/50 (4%) | 2/48 (4%) | 6/47 (13%) |
| Liver Hepatocellular adenoma or carcinoma | 3/73 (4%) | 6/50 (12%) | 6/48 (13%) | 11/47 (23%)<br>p=0.002 |
| Thyroid Follicular-cell adenoma | 0/69 (0%) | 3/50 (6%) | 1/47 (2%) | 5/46 (11%)<br>p=0.009 |

[a] Source: NTP, 1980a

[b] p-values calculated using the Fisher Exact Test.

## TABLE 11-14

### Promoting Effect of 2,3,7,8-TCDD on Hepatocarcinogenesis by a Single Dose of Diethylnitrosamine (DEN) and Partial Hepatectomy (PH)[a,b]

| Group No. | Treatment | N[c] | No. of Enzyme-Altered foci per $cm^3$ of Liver | Percent Liver Volume Which is Enzume-Altered foci | Number of Rats with Carcinoma |
|---|---|---|---|---|---|
| 1 | PH + DEN | 4 | 346 ± 65 | 5.0 | 0 |
| 2 | PH + TCDD (low dose) | 5 | 46 ± 15 | 0.1 | 0 |
| 3 | PH + TCDD (high dose) | 5 | 76 ± 20 | 0.1 | 0 |
| 4 | PH + Phenobarbital | 6 | 138 ± 40 | 0.1 | 0 |
| 5 | PH + DEN + TCDD (low dose) | 5 | 1582 ± 300 | 7.8 | 0[d] |
| 6 | PH + DEN + TCDD (high dose) | 7 | 1280 ± 40 | 35.0 | 5/7[e] ($p=0.0075$)[f] |
| 7 | PH + DEN + Phenobarbital | 4 | 1510 ± 185 | 5.0 | 2 |

[a] Source: Pitot et al., 1980

[b] Female rats (200 g) were intubated where shown with DEN. Seven days later TCDD (injected subcutaneously) or phenobarbital (0.05% in the diet) administration was begun and continued for 28 weeks at which time the animals were sacrificed and the livers examined. The low and high doses of TCDD were 0.14 and 1.4 µg/kg/2 weeks, respectively, administered subcutaneously. DEN was given at a dose of 10 mg/kg. See text for further details.

[c] Denotes the number of animals used in each group.

[d] Three rats showed "neoplastic nodules."

[e] One rat showed a "neoplastic nodule."

[f] p-value calculated using the Fisher Exact Test.

The animals in groups 1, 5, 6 and 7 received diethylnitrosamine (DEN). The rats in group 1 were then maintained on a standard laboratory diet for 32 weeks. The rats in groups 2 and 3 received no DEN, but starting 1 week after hepatectomy received biweekly subcutaneous injections of 0.14 or 1.4 µg/kg of 2,3,7,8-TCDD in corn oil for a period of 28 weeks (2,3,7,8-TCDD was 98.6% pure and provided by Dow Chemical Co.). Groups 5 and 6 received DEN, and 1 week later were initiated on a regimen of 14 biweekly injections of 0.14 and 1.4 µg/kg of 2,3,7,8-TCDD. The animals in group 4 received 0.05% sodium phenobarbital in the diet starting 1 week after partial hepatectomy for 28 weeks, and the animals in group 5 received DEN and 1 week later were also administered 0.05% sodium phenobarbital in the diet for the duration of the experiment. At the end of the experiment, rats were killed and sections of the liver were removed and frozen on solid $CO_2$. Serial sections of the frozen blocks of liver were cut and stained consecutively for glucose-6-phosphatase (G6Pase), canalicular ATPase, glutamyl transpeptidase (GGTase) with hematoxylin and eosin. The number of enzyme-altered foci were determined from photographs of histochemically stained sections. Hepatocarcinomas were diagnosed by standard histopathological criteria.

The results presented in Table 11-14 showed that the number of foci with single enzyme changes, the number of foci with multiple enzyme changes, and the total liver volume, substantially increased with the administration of 2,3,7,8-TCDD. No carcinomas were detected in four rats treated with DEN only, but five of seven rats treated biweekly with 2,3,7,8-TCDD at 1.4 µg/kg in addition to DEN had hepatocellular carcinomas, and six of seven rats had hepatocellular carcinomas or hepatocellular neoplastic nodules with a statistical significance ($p=0.0075$). Three of five rats treated biweekly with 2,3,7,8-TCDD at 0.14 µg/kg in addition to DEN had hepatocellular neoplastic nodules (p=0.083). Rats receiving only 2,3,7,8-TCDD after partial hepatectomy showed no significant increase in enzyme-altered foci and no neoplasia.

The results of this study provide evidence that 2,3,7,8-TCDD acts as a potent promoter in this two-stage model of hepatocarcinogenesis, causing increased neoplasia and increases in enzyme-altered foci at exceedingly low levels.

11.1.1.6.2. National Toxicology Bioassay Program Skin Painting Study in Mice (1980b) -- This cancer bioassay of 2,3,7,8-TCDD for possible carcinogenicity in Swiss-Webster mice was tested by the Illinois Institute of Technology under a contract sponsored by NCI. In this study, groups of 30 male and female Swiss-Webster mice were used. 2,3,7,8-TCDD in acetone suspension was applied to the skin of mice 3 days/week for 104 weeks. Male mice received 0.001 µg 2,3,7,8-TCDD per application, and the female mice received 0.005 µg 2,3,7,8-TCDD per application.

In another experiment, the same number of animals were pretreated with one application of 50 µg 7,12-dimethylbenz(l)anthracene (DMBA*) in 0.1 mℓ acetone 1 week before 2,3,7,8-TCDD application was initiated. Forty-five mice of each sex received 0.1 mℓ acetone 3 times/week and 30 animals of each sex were used as untreated controls; no DMBA control was used.

In the male and female groups of mice treated with 2,3,7,8-TCDD or 2,3,7,8-TCDD following a single application of DMBA, mean body weights were not affected as compared with the vehicle controls. Mean body weights of treated and vehicle control groups of females were lower than those of untreated controls. Mean body weights of males were less than that of untreated controls.

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*DMBA obtained from K and K Laboratories (Cleveland, Ohio). Its purity was not evaluated by NCI, but was stated by the manufacturer to be at least 95%.

The results of histopathologic diagnosis are shown in Table 11-15. The results show that 2,3,7,8-TCDD induced statistically significant ($p<0.05$) increases of fibrosarcoma in the integumentary systems of female mice treated with 2,3,7,8-TCDD alone and 2,3,7,8-TCDD following a single initial application of DMBA.

11.1.1.6.3. Berry et al. Skin Painting Study in Mice (1978, 1979) -- Berry et al. (1978) applied 2,3,7,8-TCDD in acetone solution at 0.1 µg/mouse twice weekly for 30 weeks to the skin of 30 female Charles River CD-1 mice after initiation with a single dermal application of the known skin carcinogen DMBA in acetone. After 30 weeks of promotion with 2,3,7,8-TCDD, no papillomas were observed on the DMBA-initiated mice. In the positive controls, DMBA-initiated mice were treated with 12-O-tetradecanoyl-phorbol-13-acetate (TPA) for 30 weeks; 92% of these mice developed tumors.

Berry et al. (1979) also studied the effects of treatment with 2,3,7,8-TCDD and 7,12-dimethylbenz(a)anthracene (DMBA) in a two-stage tumorigenesis bioassay in mouse skin. In this study, tumors on the shaved skin of female CD-1 mice were initiated by topical application of DMBA and were promoted with TPA. Pretreatment with 2,3,7,8-TCDD markedly inhibited the initiation of tumors by DMBA. The effects were greatest when 2,3,7,8-TCDD was applied 3-5 days before initiation and were negligible when it was applied only 5 minutes before initiation. The inhibition was almost complete (94-96%) when a single dose of 1 µg of 2,3,7,8-TCDD/mouse was applied, but was only slightly less effective (89%) when the dose was increased to 10 µg/mouse.

## TABLE 11-15

### Incidence of Primary Tumors in Mice Administered 2,3,7,8-TCDD or 2,3,7,8-TCDD Following DMBA by Dermal Application[a]

| Type of Tumors | Vehicle Control | Dose Levels[b]<br>TCDD | <br>DMBA (50 µg) plus TCDD |
|---|---|---|---|
| | | MALE | |
| Integumentary system | | 0.001 µg x 3/weeks | 0.001 µg x 3/weeks |
| Fibrosarcoma | 3/42 (7%) | 6/28 (21%)<br>p=0.08 | 6/30 (20%)<br>p=0.10 |
| | | FEMALE | |
| | | 0.005 µg x 3/weeks | 0.005 µg x 3/weeks |
| Fibrosarcoma | 2/41 (5%) | 8/27 (30%)<br>p=0.007 | 8/29 (28%)<br>p-0.010 |

[a] Source: NTP, 1980b

[b] p-value calculated using the Fisher Exact Test.

The time course of the inhibitory effects was closely parallel to the time course of induction of arylhydrocarbon hydroxylase in the skin of the mice. It was also associated with substantial reduction in the covalent binding of the DMBA metabolite to DNA and RNA, but with no change in their binding to protein.

The same authors also reported inhibitory effects of 2,3,7,8-TCDD on the initiation of mouse skin tumors by benzo(a)pyrene (BaP), although the effect was not as great (maximum 65%) with BaP as with DMBA.

11.1.1.6.4. Cohen et al. Skin Painting Study in Mice (1979) -- Cohen et al. (1979) showed that pretreatment of mice with dermally applied 2,3,7,8-TCDD resulted in the inhibition of skin tumor induction by subsequent treatment with DMBA and BaP. The inhibition of skin carcinogenesis by BaP in mice after pretreatment with 2,3,7,8-TCDD was associated with an increase in covalent binding of BaP metabolites to DNA, RNA and protein (in contrast to the results with DMBA, which showed a reduction in binding to DNA and RNA). However, the BaP metabolites that were bound to DNA and RNA in mice pretreated with 2,3,7,8-TCDD differed from those in untreated mice. In particular, pretreatment with 2,3,7,8-TCDD markedly reduced the formation of the presumptive ultimate carcinogenic metabolite of BaP, 7,8-diol-9,10-epoxy-BaP and its covalent binding with guanosine in DNA.

11.1.1.6.5. Kouri et al. Mouse Study (1978) -- This study was designed as an investigation of the cocarcinogenic activity of 2,3,7,8-TCDD administered to mice in conjunction with subcutaneous administration of 3-methylcholanthrene (3-MC). Two inbred strains in mice, C57BL/6Cum (abbreviated B6) and DBA/2Cum (abbreviated D2), were used. These strains are responsive and nonresponsive, respectively, to the induction of aryl hydrocarbon hydroxylase (AHH) by 3-MC.

Groups of mice of both sexes were injected subcutaneously at 4-6 weeks of age with either 150 μg of 3-MC dissolved in trioctanoin or with trioctanoin alone. Some groups were also injected with 2,3,7,8-TCDD dissolved in p-dioxane, either simultaneously with the administration of 3-MC or 2 days earlier. Two doses of 2,3,7,8-TCDD (1 μg/kg and 100 μg/kg) were used, and the effects of both intraperitoneal and subcutaneous injections were investigated. Two sets of experiments involving 29 groups of mice were conducted ~1 year apart (Tables 11-16 and 11-17).

After treatment, the mice were observed for 36 weeks, during which time they were palpated weekly for the presence of tumors; latency was calculated when the subcutaneous tumors became 1 cm in diameter. Only tumors characterized histologically as fibrosarcomas at the site of inoculation were considered. It is unclear whether or not these were the only tumor types observed. The term "carcinogenic index" used by the authors was defined as the percentage of tumor incidence 8 months after treatment divided by the average latency in days multiplied by 100. No details were given of the number of animals in each group at the start of each experiment, but the numbers dying in the first 28 days and the numbers at risk (surviving 36 weeks) were tabulated. The results of this study are shown in Tables 11-16 and 11-17.

No subcutaneous tumors were observed in controls or in mice treated with 2,3,7,8-TCDD alone. In B6 (responsive) mice, the administration of 2,3,7,8-TCDD did not significantly enhance the induction of tumors by 3-MC. However, in both experiments involving D2 (nonresponsive) mice, the administration of 2,3,7,8-TCDD simultaneously with 3-MC appeared to enhance the carcinogenic response. The "carcinogenic index" increased from 1-6 in groups treated with 3-MC alone to 14 in the group treated subcutaneously

TABLE 11.16

Effects of Intraperitoneal Administration of 2,3,7,8-TCDD on 3-MC-Initiated Subcutaneous Tumors[a]

| Inbred Strain | Treatment | | No. of Mice Dying Because of Treatment[b] | No. of Mice at Risk for Tumors[c] | No. of Mice with Tumors[d] | % of Mice with Tumors | Average Latency (days) | Carcinogenic Index[e] |
|---|---|---|---|---|---|---|---|---|
| | -2 Days | 0 Days | | | | | | |
| B6 | i.p. p-dioxin | s.c. trioctanoin | 1 | 39 | 0 | 0 | | |
| | i.p. TCDD (100 µg/kg) | s.c. trioctanoin | 20 | 27 | 0 | 0 | | |
| | None | s.c. 3-MC | 1 | 36 | 29 | 81 | 125 | 65 |
| | None | i.p. TCDD (100 µg/kg) | 20 | 30 | 0 | 0 | | |
| | None | i.p. TCDD (100 µg/kg) + s.c. 3-MC | 30 | 43 | 33 | 71 | 123 | 63 |
| | None | i.p. TCDD (1 µg/kg) | 4 | 46 | 0 | 0 | | |
| | None | i.p. TCDD (1 µg/kg) + s.c. 3-MC | 6 | 27 | 27 | 100 | 132 | 76 |
| | i.p. TCDD (100 µg/kg) | s.c. 3-MC | 20 | 25 | 21 | 84 | 129 | 65 |
| | i.p. TCDD (1 µg/kg) | s.c. 3-MC | 6 | 23 | 16 | 70 | 140 | 50 |
| D2 | i.p. p-dioxane | s.c. trioctanoin | 6 | 22 | 0 | 0 | | |
| | i.p. TCDD (100 µg/kg) | s.c. trioctanoin | 24 | 25 | 0 | 0 | | |
| | None | s.c. 3-MC | 3 | 34 | 1 | 3 | 217 | 1 |
| | None | i.p. TCDD (100 µg/kg) | 30 | 38 | 0 | 0 | | |
| | None | i.p. TCDD (100 µg/kg) + s.c. 3-MC | 43 | 43 | 10 | 23 | 178 | 13[f] |
| | None | i.p. TCDD (1 µg/kg) | 5 | 48 | 0 | 0 | | |
| | None | i.p. TCDD (1 µg/kg) + s.c. 3-MC | 5 | 34 | 5 | 15 | 199 | 7 |
| | i.p. TCDD (100 µg/kg) | s.c. 3-MC | 20 | 28 | 0 | 0 | | |
| | i.p. TCDD (1 µg/kg) | s.c. 3-MC | 6 | 31 | 0 | 0 | | |

[a] Source: Kouri et al., 1978

[b] During the first 28 days following treatment.

[c] Defined as the number of mice surviving the 36-week observation period.

[d] At the end of the 36-week experiment.

[e] Percentage of incidence of tumors, divided by the average latency in days, multiplied by 100 (8).

[f] This carcinogenic index value lies outside (greater than) the 99% confidence interval (i.e., $p<0.01$) constructed from seven different studies over the past 5 years during which 150 µg of 3-MC was given s.c. to D2 mice. These studies included 295 D2 mice, the mean ± S.D. for all seven studies was a carcinogenic index of 5.43 ± 2.70.

TABLE 11-17

Effect of Intraperitoneal or Subcutaneous Administration of 2,3,7,8-TCDD Given 2 Days Before or Simultaneous With Subcutaneous Administration of 3-MC on Tumorigenesis in D2 Mice[a]

| Treatment | | No. of Mice Dying Because of Treatment | No. of Mice at Risk for Tumors | No. of Mice with Tumors | % of Mice with Tumors | Average Latency (days) | Carcinogenic Index |
|---|---|---|---|---|---|---|---|
| -2 Days | 0 Days | | | | | | |
| None | s.c. 3-MC | 0 | 30 | 3 | 10 | 177 | 6 |
| i.p. p-dioxane | s.c. 3-MC | 10 | 40 | 3 | 10 | 194 | 5 |
| i.p. TCDD (100 μg/kg) | s.c. 3-MC | 35 | 65 | 9 | 14 | 145 | 10 |
| None | i.p. p-dioxane + s.c. 3-MC | 5 | 45 | 5 | 11 | 176 | 6 |
| None | i.p. TCDD (100 μg/kg) + s.c. 3-MC | 38 | 62 | 17 | 27 | 183 | 15[b] |
| None | i.p. TCDD (1 μg/kg) + s.c. 3-MC | 22 | 78 | 8 | 10 | 162 | 6 |
| None | s.c. p-dioxane + s.c. 3-MC | 2 | 68 | 8 | 12 | 180 | 6 |
| None | s.c. TCDD (100 μg/kg) | 8 | 42 | 0 | 0 | | |
| None | s.c. TCDD (100 μg/kg) + s.c. 3-MC | 18 | 82 | 46 | 55 | 145 | 38[b] |
| None | s.c. TCDD (1 μg/kg) | 2 | 48 | 0 | 0 | | |
| None | s.c. TCDD (1 μg/kg) + s.c. 3-MC | 2 | 98 | 21 | 21 | 154 | 14[b] |

[a] Source: Kouri et al., 1978

[b] These carcinogenic index values lie outside the 99% confidence interval.

with 2,3,7,8-TCDD at 1 μg/kg, and 13-15 in the groups treated intraperitoneally with 2,3,7,8-TCDD at 100 μg/kg. The authors concluded that 2,3,7,8-TCDD acts as a cocarcinogen, possibly as an inducer of AHH at the site of inoculation.

A more appropriate statistical analysis would be a comparison of tumor incidence in 2,3,7,8-TCDD-treated groups with tumor incidence in corresponding 3-MC-treated groups within the same experiment. The results of this analysis are given in Table 11-18.

From these results, the CAG concluded that the experiment adequately demonstrated the enhancement by 2,3,7,8-TCDD of tumor induction when 2,3,7,8-TCDD was administered simultaneously with 3-MC at the higher dose (100 μg/kg). The reported results at the lower dose (1 μg/kg) are not statistically significant unless the reduction in latency is taken into account, which is difficult to do rigorously. Despite defects in reporting (failure to specify the initial number of animals in each group and to report tumor incidence by sex), the results provide evidence that 2,3,7,8-TCDD acts as a cocarcinogen. The failure of 2,3,7,8-TCDD to induce tumors when administered alone was not unexpected since only a single dose was administered and the duration of the study was very short (36 weeks).

11.1.1.6.6. Poland et al. Study (1982) -- Poland et al. (1982) described studies which indicate that genetic differences in mice affect the tumor-promoting capacity of 2,3,7,8-TCDD in the mouse skin two-stage tumorigenesis model. Both 2,3,7,8-TCDD and TPA were compared for tumor-promoting activity in DMBA-initiated HRS/J mice that were either heterozygous (hour/+) or homozygous (hour/hour) for the recessive "hairless" trait. Promotion with biweekly applications of 2 μg of TPA for 25 weeks resulted in papilloma incidences of 100 and 70% in (hour/+) and (hour/hour) mice,

## TABLE 11-18

### Incidence of Tumors in Mice Treated With 3-MC and With 3-MC and 2,3,7,8-TCDD[a]

| Experiment | Dose of TCDD (μg/kg) | Route of Administration | Tumor Incidence | | p-Value[b] |
|---|---|---|---|---|---|
| | | | TCDD and 3-MC | 3-MC | |
| 1 | 100 | intraperitoneal | 10/43 | 1/34 | p=0.01 |
| 2 | 100 | intraperitoneal | 17/62 | 5/45 | p=0.03 |
| 2 | 100 | subcutaneous | 46/82 | 5/42 | $p=3.0 \times 10^{-7}$ |
| 2 | 1 | subcutaneous | 21/98 | 5/45 | p=0.1 |

[a] Source: Kouri et al., 1978

[b] p-value calculated using the Fisher Exact Test (one-tailed).

respectively. Promotion of DMBA-initiated (hour/+) mice with 2,3,7,8-TCDD (50 ng/application for 8 weeks followed by 20 ng/application) did not result in the formation of tumors, while promotion of (hour/hour) mice resulted in both the same incidence and multiplicity of tumors as observed in TPA-promoted mice. With either DMBA or methyl-N-nitrosoguanidine (MNNG)-initiated (hour/hour) mice, the effective dose of 2,3,7,8-TCDD was ~100-fold less than TPA on a molar basis. Histologic examination of the skin showed that TPA produced both acute inflammation and hyperplasia in (hour/+) and (hour/hour) mice, while 2,3,7,8-TCDD produced hyperplasia and hyperkeratosis only in (hour/hour) mice with no inflammatory response. The lack of a 2,3,7,8-TCDD-induced inflammatory response suggested to the authors that 2,3,7,8-TCDD promoted skin papillomas in (hour/hour) mice by a mechanism different from TPA.

11.1.1.6.7. DiGiovanni et al. Study (1977, 1980) -- Investigations have also been conducted on the effects of prior or simultaneous treatment with 2,3,7,8-TCDD on the subsequent development of skin tumors by chemical carcinogens. When 2,3,7,8-TCDD (0.1 μg) was administered simultaneously with DMBA (200 nmol) to the backs of CD-1 mice in a single initiation dose, the skin papilloma incidence following promotion with TPA was nearly the same as when DMBA alone was used as the initiator (DiGiovanni et al., 1977). Although simultaneous exposure to 2,3,7,8-TCDD and DMBA did not appreciably affect tumor yield, Berry et al. (1979) demonstrated a marked 93% decrease in the incidence of DMBA-initiated tumors when CD-1 mice were pretreated 3 days before DMBA initiation with 1 μg/mouse of 2,3,7,8-TCDD. The time of treatment with 2,3,7,8-TCDD in relation to initiation was shown to be critical in the antitumorigenic effects of 2,3,7,8-TCDD (Berry et al., 1979;

DiGiovanni et al., 1979a, 1980), as shown in Figure 11-1. Maximum tumor inhibition of between 86 and 94% occurred when pretreatment was between 1 and 5 days before initiation. If pretreatment was 10 days before DMBA initiation, the tumor yield was decreased by 78%, while 2,3,7,8-TCDD treatment 5 minutes before or 1 day after DMBA initiation had no effect on tumor yield. There was some indication of an inverse relationship between the pretreatment dose of 2,3,7,8-TCDD (3 days before DMBA initiation) and the incidence of tumors. 2,3,7,8-TCDD doses of 0.0, 0.01, 0.1 and 2 µg/mouse resulted in decreased tumor yields, respectively, of 0, 83, 92 and 96% (DiGiovanni et al., 1979a). Also under similar experimental conditions Cohen et al. (1979) observed a 75% decrease in the incidence of skin tumors in Sencar mice pretreated with 1 µg of 2,3,7,8-TCDD 3 days before initiation by DMBA.

DiGiovanni et al. (1980) investigated the antitumorigenic effect of 2,3,7,8-TCDD in CD-1 mice with chemical carcinogens other than DMBA (see Figure 11-1). As observed with DMBA, exposure to 2,3,7,8-TCDD 3 days before initiation with either benzo(a)pyrene (BaP) or 3-MC resulted in a decrease in tumor yield as compared with acetone-pretreated animals; however, pretreatment with 2,3,7,8-TCDD 5 minutes before or 1 day after initiations was ineffective in changing the tumor yield. The maximum decrease in tumor production was 86 and 57%, respectively, for BaP and 3-MC initiated mice. A different temporal relationship was observed in the ability of 2,3,7,8-TCDD to inhibit tumor formation by BaP-diol-epoxide as compared with the previously studied polyaromatic hydrocarbons (PAH). When 2,3,7,8-TCDD was applied 3 days or 5 minutes before, or 1 day after initiation with BaP-diol epoxide, there was an 81.5 and 49% decrease in tumor yield. Examination of PAH metabolism in the skin of mice treated with 2,3,7,8-TCDD showed a

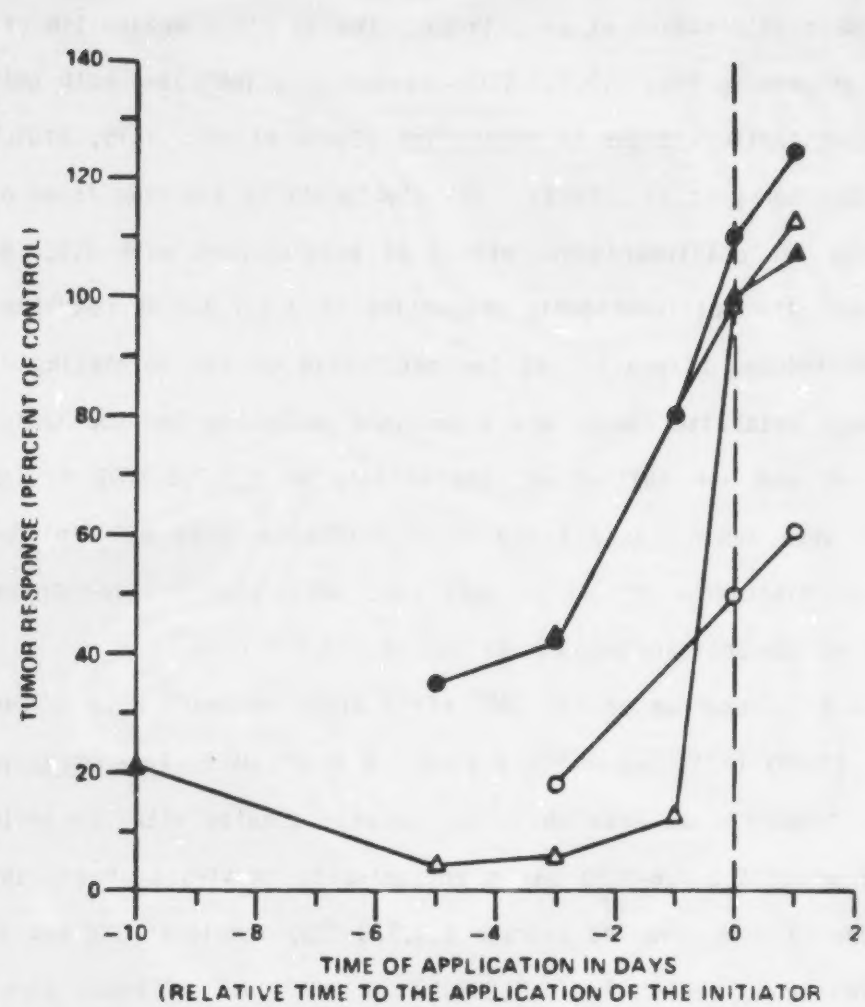

FIGURE 11-1

Time-Dependent Inhibition by 2,3,7,8-TCDD of Tumor Initiation

Summary of the time-dependent inhibitory effect of 2,3,7,8-TCDD on tumor initiation by DMBA (Δ), BaP (o), 3-MC (▲) and BaP-diol-epoxide (●). Animals were initiated with 10 nmol DMBA, 100 nmol BaP, 100 nmol 3-MC and 200 nmol BaP-diol-epoxide and promoted 1 week later with twice weekly application of TPA.

21-fold increase in aryl hydrocarbon hydroxylase (AHH) activity 72 hours after treatment (DiGiovanni et al., 1980). The in vitro metabolism of DMBA by dermal homogenates from 2,3,7,8-TCDD-treated mice indicated both qualitative and quantitative changes in metabolism (Cohen et al., 1979; DiGiovanni et al., 1979a; Berry et al., 1979). The similarity in the time frame of AHH induction and the antitumorigenic effect of pretreatment with 2,3,7,8-TCDD suggested that the antitumorigenic properties of 2,3,7,8-TCDD resulted from 2,3,7,8-TCDD induced alteration in the metabolism of the initiating chemical. Although metabolic change was a possible mechanism for the inhibition of DMBA, 3-MC and BaP initiation, the ability of 2,3,7,8-TCDD to inhibit tumor yield when administered 1 day after initiation with BaP-diol-epoxide indicated by DiGiovanni et al. (1980) that more than one mechanism may participate in the anticarcinogenic effect of 2,3,7,8-TCDD.

11.1.1.6.8. Cockerham et al. 1980 Field Study on Beach Mice -- Cockerham et al. (1980) performed a field study on beach mice, Peramyscus polienotus, that inhabited an area which was heavily treated with the herbicide 2,4,5-T, of which 2,3,7,8-TCDD was a contaminant. Analysis of the soil in the contaminated area revealed average 2,3,7,8-TCDD levels of 150 ppt at the surface. Measured levels of 2,3,7,8-TCDD in the liver of beach mice from the contaminated area were determined to be 1300 ppt in males and 960 ppt in females. Detection of 2,3,7,8-TCDD in the liver indicates that the compound was absorbed; however, since seeds in the area did not contain 2,3,7,8-TCDD, it was believed that the animals ingested the compound from contaminated dust while grooming. In the 10 male and 5 female animals captured in the contaminated area, there were no histopathologic differences, including neoplastic lesions, observed in the liver as compared with 9 male and 6 female mice captured in a noncontaminated area. The only observed difference in the two groups of mice was a statistically significant (95% confidence) increase in liver-to-body weight ratios. The authors back-calculated from the 2,3,7,8-TCDD levels of the liver and estimated a daily 2,3,7,8-TCDD dose of 0.0012 μg/kg bw. It was noted that this exposure was much lower than the exposures used in laboratory studies to produce tumors.

### 11.1.2. Studies Using HxCDD.

11.1.2.1. NATIONAL TOXICOLOGY BIOASSAY PROGRAM (ORAL) STUDY IN RATS AND MICE (NTP, 1980d) -- Although 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD have not been tested individually for carcinogenicity, the NTP has performed a chronic bioassay in both Osborne-Mendel rats and B6C3F1 mice to determine the carcinogenicity of a mixture of 1,2,3,6,7,8- and 1,2,3,7,8,9-HxCDD (NTP, 1980d). The mixture consisted of 31% of the 1,2,3,6,7,8-HxCDD congener and 67% of the 1,2,3,7,8,9-HxCDD congener, with a total HxCDD purity of 98%. The following impurities were detected in HxCDD used for this bioassay: PeCDD, 0.04%; TCDD, 0.09%±0.03%; TriCDD, 0.004%; DCDD, 0.004% and Bromo PeCDD, <0.004%. The specific isomers of these impurities were not identified. The compound was protected from light during storage, and every 3 months a stock acetone suspension was prepared. The working solution was administered to the test animals in corn oil-acetone (9:1) by gavage 2 times/week. All treated groups consisted of 50 animals of each sex, while the control groups, both vehicle and untreated controls, consisted of 75 animals of each sex. The male and female rats, and the male mice received HxCDD doses of 0.0, 1.25, 2.5 and 5 μg/kg/week, and the female mice received doses of 0.0, 2.5, 5.0 and 10 μg/kg/week. Treatment was continued for 104 weeks followed by a 3- to 4-week observation period. Complete necropsies, including extensive histologic examinations, were performed on animals at the time of natural death, when moribund or at the termination of the study.

A decrease in body weight gain was seen at the two higher exposure levels. A dose-related "toxic hepatitis" that was noninflammatory and consisted of degenerative changes in the liver, eosinophilic foci of cellular alteration, mild fibrosis and bile duct hyperplasia was also observed. Cytomegaly and lipidosis were included in these degenerative changes. The only neoplastic lesions that appeared to be treatment-related were neoplastic nodules of the liver and hepatocellular carcinomas (Table 11-19). The combined incidences of these tumors in male rats were 0/74, 0/49, 1/50 and 4/48, while in female rats the incidences were 5/75, 10/50, 12/50 and 30/50 for the control, low-, medium- and high-dose groups, respectively. The incidence of liver tumors in male rats showed a positive dose-related trend by the Cochran-Armitage test; the incidence in the high-dose male rat group was statistically different from the control group by the Fisher exact test ($p=0.022$) but the requirements by NTP for overall significance were not met based on the Bonferroni inequality. The NTP thus concluded that the evidence for the carcinogenicity of HxCDD in male rats was inconclusive. In female rats, the Cochran-Armitage test was significant at $p<0.001$, and the liver tumor incidence of the high-dose animals was significantly ($p<0.001$) different from that of the control group, as well as with the mid-dose group ($p=0.006$).

Subsequent to the release of the NTP gavage study of HxCDD in rats and mice (NTP, 1980d), several pathologists reevaluated the microscopic slide material of the female rats. These reviews resulted from a report by Squire (1983) which stated that many of the entities diagnosed as tumors by NTP were actually nonneoplastic regenerative nodules; but his report concluded that the HxCDD bioassay still provided evidence of a weak hepatocarcinogenic

## TABLE 11-19

### Liver Tumor Incidences in Male and Female Osborne-Mendel Rats Administered HxCDD for 104 Weeks[a]

| Diagnoses | Treatment Group<br>Untreated Control | Vehicle Control | Low Dose | Mid Dose | High Dose |
|---|---|---|---|---|---|
| **MALE** | | | | | |
| Neoplastic nodule (NN) | 2/75[b] | 0/74 | 0/49 | 1/50 | 3/48 |
| Hepatocellular carcinoma (HC) | 0/75 | 0/74 | 0/49 | 0/50 | 1/48 |
| Combined NN + HC | 2/75 | 0/74 | 0/49 | 1/50 | 4/48<br>p=0.002[c] |
| **FEMALE** | | | | | |
| Neoplastic nodule (NN) | 1/73 | 5/75 | 10/50<br>p=0.026 | 12/50<br>p=0.006 | 30/50<br>$p=6.94\times10^{-11}$ |
| Hepatocellular carcinoma (HC) | 0/74 | 0/75 | 0/50 | 0/50 | 4/50<br>p=0.024 |
| Combined NN + HC | 1/73 | 5/75 | 10/50<br>p=0.026 | 12/50<br>p=0.006 | 30/50<br>$p=6.94\times10^{-11}$ |

[a] Source: Adapted from NTP, 1980c

[b] $\text{Incidence} = \dfrac{\text{No. of rats with lesion}}{\text{No. of rats examined microscopically}}$

[c] p-values calculated using the Fisher Exact Test.

effect in rats and mice. Drs. R. Schueler and B. Haberman also reported discrepancies in the diagnoses of liver tumors from the NTP gavage study. Their findings were reported in an internal U.S. EPA memorandum from CAG to J. Bellin (U.S. EPA, 1983b) with an attached report prepared by Dr. R. Schueler, Research Pathology Associates, Inc. (Schueler, 1983). Finally, Dr. E. McConnel of NTP requested that Dr. P. Hildebrandt of Tracor-Jitco, Inc., review the microscopic slides of the HxCDD bioassay (gavage) in the female rat; his findings (Hildebrandt, 1983) agreed closely with those of Drs. Schueler and Haberman. Dr. Hildebrandt's findings (Table 11-20), although not as statistically significant as the original NTP findings, still confirmed that the HxCDD mixture administered by gavage produced an increased incidence of liver tumors in treated female rats as compared with control animals, as well as an increase in "toxic hepatitis."

In mice there were no gross signs of HxCDD toxicity; however, as observed in rats, there was a dose-related incidence of "toxic hepatitis" consisting of degenerative liver changes and/or necrosis associated with cellular infiltration and mild fibrosis. The only neoplastic changes that were treatment-related were increases in hepatocellular adenomas and carcinomas (Table 11-21). The adenomas were characterized as groups of cells with a uniform cell type that did not conform to the lobular architecture and which caused compression of the surrounding normal liver, while the carcinomas contained cells with greater histologic deviations, disorganized growth and more cells in mitosis. A few liver tumors in control and dosed groups metastasized to the lungs. The incidence of hepatocellular adenomas or carcinomas were 15/73, 14/50, 14/49 and 24/48 in male mice, and 3/73, 4/48, 6/47 and 10/47 in female mice of the control, low-, medium- and high-dose groups, respectively. In both male and female mice, the liver tumor

### TABLE 11-20

### Liver Tumor Incidences in Female Osborne-Mendel Rats Administered HxCDD by Gavage for 104 Weeks[a]

| Diagnoses | Untreated Control | Vehicle Control | µg/kg/week | | |
|---|---|---|---|---|---|
| | | | Low Dose 1.25 | Mid Dose 2.5 | High Dose 5 |
| Neoplastic nodule (NN) | 1/73[b] | 2/75 | 5/50 | 7/50<br>$p=0.02$[c] | 16/50<br>$p=6.0\times10^{-6}$ |
| Hepatocellular carcinoma (HC) | 0/73 | 0/75 | 0/50 | 0/50 | 2/50 |
| Combined NN + HC | 1/73 | 2/75 | 5/50 | 7/50<br>$p=0.02$ | 18/50<br>$p=7.3\times10^{-7}$ |

[a] Source: Adapted from Hildebrandt, 1983

[b] Incidence = $\frac{\text{No. of rats with lesion}}{\text{No. of rats examined microscopically}}$

[c] p-values calculated using the Fisher Exact Test.

## TABLE 11-21

### Liver Tumor Incidences in Male and Female B6C3F1 Mice Administered HxCDD by Gavage for 104 Weeks[a]

| Diagnoses | Treatment Group | | | | |
|---|---|---|---|---|---|
| | Untreated Control | Vehicle Control | Low Dose | Mid Dose | High Dose |
| **MALE** | | | | | |
| Hepatocellular adenoma (HA) | 15/75[b] | 7/73 | 5/50 | 9/49 | 15/48<br>$p=0.003$[c] |
| Hepatocellular carcinoma (HC) | 12/75 | 8/73 | 9/50 | 5/49 | 9/48 |
| Combined HA + HC | 27/75 | 15/73 | 14/50 | 14/49 | 24/48<br>$p=7.33\times10^{-4}$ |
| **FEMALE** | | | | | |
| Hepatocellular adenoma (HA) | 2/74 | 2/73 | 4/48 | 4/47 | 9/47<br>$p=0.003$ |
| Hepatocellular carcinoma (HC) | 0/74 | 1/73 | 0/48 | 2/47 | 2/47 |
| Combined HA + HC | 2/74 | 3/73 | 4/48 | 6/47 | 10/47<br>$p=0.004$ |

[a] Source: Adapted from NTP, 1980c

[b] $\text{Incidence} = \frac{\text{No. of rats with lesion}}{\text{No. of rats examined microscopically}}$

[c] p-values calculated using the Fisher Exact Test.

incidence showed a significant dose-related trend by the Cochran-Armitage test, and the incidence of tumors in the high-dose group was significantly higher than the incidence in the control group by the Fisher exact test.

The obvious question was raised concerning the presence of tetrachlorodibenzo-p-dioxin as an impurity (0.09%) in the test material, which may have contributed to the observed liver tumor incidence. The analysis presented in Table 11-22 shows that the calculated 95% upper-limit liver cancer response due to 0.09% TCDD impurity is so low as compared with the observed liver cancer response due to HxCDD in this cancer bioassay study, it is reasonable to conclude that the impurity in the test material did not contribute significantly to the observed carcinogenic response for HxCDD.

McGaughy and Rispin (1985) made the following comments regarding the three documents listed below, which evaluated issues that have been raised with respect to the NCI/NTP HxCDD carcinogenicity bioassay on rats and mice:

1. The responses outlined by the Office of Health and Environmental Assessment (OHEA) were prepared for presentation to EPA's Science Advisory Board on November 28, 1984.
2. The document entitled "Response to Comments" was prepared by Agency staff and a consultant pathologist.
3. A memorandum from Dr. John Doull, member of EPA's Science Advisory Board, concerning the HxCDD audit by Dr. G. Schoenig.

The above documents respond to questions that were raised concerning many aspects of the bioassay study. These questions relate, for example, to allegations of problems in:

- test procedures, such as problems in preparation of the test material; flaws in methods of administration; flaws in recordkeeping procedures and practices.
- pathology practices, such as non-uniform and substandard tissue harvesting practices; non-uniform histologic procedures; bias in histology review; and deficiencies in correlation between gross and microscopic observations.

## TABLE 11-22

## Liver Tumor Response for HxCDD (Observed) and TCDD Contaminant (Calculated)

| Animal | HxCDD Dose (μg/kg/week) | Liver Cancer Response Observed | 0.09% TCDD Contaminant Dose[a] (μg/kg/week) | Liver Cancer Response Calculated 95% Upper Limit |
|---|---|---|---|---|
| Rat (OM) | | | | |
| Male | 5 | 4/48[b] | 0.0045 | TCDD has shown no effect in NCI study |
| Female | 5 | 18/50[c] | 0.0045 | 0.02/50[d] |
| Mouse (B6C3F1) | | | | |
| Male | 5 | 24/48[e] | 0.0045 | 0.20/48[d] |
| Female | 10 | 10/47[f] | 0.009 | 0.22/47[e] |

[a] It is assumed that all of the contaminant is 2,3,7,8-TCDD.

[b] NTP reviewed

[c] Re-evaluation by Hildebrandt (see Table 11-35)

[d] Based on response in NCI 2,3,7,8-TCDD study; see Table B-10.

[e] Based on response in NCI 2,3,7,8-TCDD study; see Table B-11.

[f] Based on response in NCI 2,3,7,8-TCDD study; see Table B-12.

- alleged bias in the above practices with respect to treated and control animals.

- pathology interpretation: disagreement in conclusions reached by different pathologists.

From our review of these documents we conclude that there were indeed some procedural flaws during the in-life portion of the study, and there were minor recordkeeping problems. The management of a two-year rodent study is a very complex undertaking. It is therefore not surprising that the procedural and recordkeeping deficiencies highlighted by the two audits occurred. They do not invalidate the study.

The detailed review by Agency staff and Dynamac Corporation of Dr. Schoenig's findings concerning room bias did not substantiate his allegations. Similarly, review of Dr. Schoenig's criticism of the histologic practices did not reveal meaningful deficiencies in tissue harvesting, preparation of microscopic slides, and histologic diagnoses.

Differences in interpretation among pathologists have previously been addressed by the Agency (see the OHEA document attached hereto). The slight differences in interpretation among the different pathologists do not alter the conclusion as to the carcinogenic potential of HxCDD.

We conclude that the HxCDD bioassay is valid, and that it can appropriately be used for the assessment of the carcinogenic potential of HxCDD.

Under the test conditions of this bioassay, the 1:2 mixture of 1,2,3,7,8- and 1,2,3,7,8,9-HxCDD was carcinogenic, as indicated by a statistically significant increased incidence in tumors of the liver in female rats and in both male and female mice, and by a borderline liver tumor response in male rats.

11.1.2.2. NATIONAL TOXICOLOGY BIOASSAY PROGRAM SKIN-PAINTING STUDY IN MICE (NTP, 1980b,c) -- Both 2,3,7,8-TCDD (NTP, 1980b) and a 2:1 mixture of 1,2,3,6,7,8- and 1,2,3,7,8,9-HxCDD (NTP, 1980c) have been tested in mice for tumorigenic potential by dermal application. These studies were conducted under the NTP and the description of the chemicals used was the same as previously presented in the discussion of NTP (1980a,d). There was no information found in the literature searched on the tumorigenic effect of 1,2,3,7,8-PeCDD following dermal exposure. The tumorigenic response after chronic dermal exposure to HxCDD was presented in Table 11-23.

In both NTP bioassays (1980b,c), groups of 30 male and 30 female Swiss-Webster mice were treated with 100 µℓ of a solution of the test compound in acetone 3 times/week for 104 weeks. Groups of 45 animals were employed as vehicle controls, and 2 groups of 15 animals were used as untreated controls. The concentration of 2,3,7,8-TCDD used resulted in a dose of 0.01 µg/application in male mice and 0.005 µg/application in female mice; the concentration of HxCDD used resulted in a dose of 0.005 µg/application for the initial 16 weeks of the study, followed by a subsequent increase to 0.01 µg/application for the remainder of the study. Subchronic toxicity studies used to define the dose levels for the chronic bioassay indicated that all the doses used resulted in some liver damage but no increase in mortality. In the chronic study, animals were killed when moribund at the termination of the study and examined for gross tumors. Microscopic examinations were also made of all major organs.

In mice exposed to 2,3,7,8-TCDD (NTP, 1980b), there was no treatment-related difference in body weight of either sex between exposed animals and control groups; however, male mice treated with 2,3,7,8-TCDD had a significant shortening of lifespan. Nontumorigenic hepatic lesions were observed in treated female mice; no mention was made of these lesions occurring in male mice. The only tumors that were treatment-related were integumentary system fibrosarcomas, with tumors developing on or near the site of application. The incidence of these tumors in male mice was 3/42 and 6/28, and in female mice the incidences were 2/41 and 8/27, respectively, for the vehicle control groups and the treated animals. Only the tumor incidence in female

## TABLE 11-23

### Carcinogenicity Bioassays of 2,3,7,8-TCDD and HxCDD by Dermal Application to Mice[a]

| Compound | Sex | Dose[b] | Duration of Exposure | Target Organ | Tumor Type | Tumor Incidence |
|---|---|---|---|---|---|---|
| 2,3,7,8-TCDD | M | 0.01 µg/application | 104 weeks | integumentary system | fibrosarcoma | 6/28 |
|  | M | 0.0 µg/application (vehicle control) | 104 weeks | integumentary system | fibrosarcoma | 3/42 |
|  | M | 0.0 µg/application (untreated control) | NA | integumentary system | fibrosarcoma | 0/28 |
| 2,3,7,8-TCDD | F | 0.005 µg/application | 104 weeks | integumentary system | fibrosarcoma | 7/28 |
|  | F | 0.0 µg/application (vehicle control) | 104 weeks | integumentary system | fibrosarcoma | 2/41 |
|  | F | 0.0 µg/application (untreated control) | NA | integumentary system | fibrosarcoma | 1/27 |
| HxCDD | M | 0.01 µg/application[c] | 104 weeks | lung | alveolar/ bronchiolar carcinoma | 5/30 |
|  | M | 0.0 µg/application (vehicle control) | 104 weeks | lung | alveolar/ bronchiolar carcinoma | 1/41 |

TABLE 11-23 (cont.)

| Compound | Sex | Dose[b] | Duration of Exposure | Target Organ | Tumor Type | Tumor Incidence |
|---|---|---|---|---|---|---|
| HxCDD (cont.) | M | 0.0 μg/application (untreated control) | NA | lung | alveolar/ bronchiolar carcinoma | 4/28 |
| HxCDD | F | 0.01 μg/application[c] | 104 weeks | skin | fibrosarcoma | 4/27 |
|  | F | 0.0 μg/application (vehicle control) | 104 weeks | skin | fibrosarcoma | 2/41 |
|  | F | 0.0 μg/application (untreated control) | NA | skin | fibrosarcoma | 0/30 |

[a] Source: NTP, 1980b,c

[b] The compound was applied 3 times/week in 100 μℓ of acetone.

[c] For the initial 16 weeks of the study, the dose was 0.005 μg/application.

NA = Not applicable

mice was statistically (p=0.007) greater than control values; however, life table analyses indicated that the time to tumor was shorter in both male and female treated mice. The incidence of tumors in untreated and vehicle control groups was similar.

In the bioassay of HxCDD (NTP, 1980c), no gross or nonneoplastic histologic effects associated with treatment were observed. Although there was a slight increase in the incidence of skin fibrosarcomas in female mice, this increase was significant in comparison with the vehicle control group, but not significantly different from the untreated control group. The opposite occurred with the incidence of alveolar/bronchiolar carcinomas of the lung in male mice, which was significantly elevated in comparison with untreated but not vehicle-treated controls. It was concluded that although dermal exposure to 2,3,7,8-TCDD resulted in a carcinogenic response in both male and female Swiss-Webster mice, dermal exposure to a mixture of 1,2,3,7,8-TCDD and 1,2,3,7,8,9-HxCDD did not result in a carcinogenic response under the conditions of this bioassay. A summary of the carcinogenicity bioassays is given in Table 11-24.

11.1.3. **Summary of Animal Carcinogenicity.** In a preliminary study by Van Miller (1977a,b), 2,3,7,8-TCDD was tested for carcinogenicity following oral administration to rats. At the five highest dietary levels, 0.005, 0.05, 0.5, 1.0 and 5.0 ppb, which allowed long-term survival of the animals, an increased incidence of total tumors was observed. In animals at an exposure level of 0.001 ppb and in the control animals there were no tumors. This study, however, provides only suggestive evidence of a carcinogenic response since no increase in site-specific tumors was detected and the group sizes, ~10 animals/group, were too small for an assessment of a treatment-related response. In a second, more extensive study by Kociba et al. (1978a) a positive carcinogenic response was detected. In this study the estimated

## TABLE 11-24

### Carcinogenicity Bioassays of PCDD Administration by the Oral and Dermal Route

| Exposure Route/ Compound | Species/Strain | Sex | Dose or Exposure | Duration of Treatment | Duration of Study | Vehicle | Tumor Type | Tumor Incidence | Reference |
|---|---|---|---|---|---|---|---|---|---|
| Gavage/ 2,3,7,8-TCDD | rats/ Osborne-Mendel | M | 0.0 µg/kg/week | 104 weeks | 105 weeks | corn oil-acetone (9:1) | follicular-cell adenomas or carcinoma of the thyroid | 1/69 | NTP, 1980a |
| | | | 0.1 µg/kg/week | 104 weeks | 107 weeks | corn oil-acetone (9:1) | follicular-cell adenomas or carcinoma of the thyroid | 5/48 | |
| | | | 0.05 µg/kg/week | 104 weeks | 107 weeks | corn oil-acetone (9:1) | follicular-cell adenomas or carcinoma of the thyroid | 8/50 | |
| | | | 0.5 µg/kg/week | 104 weeks | 105 weeks | corn oil-acetone (9:1) | follicular-cell adenomas or carcinoma of the thyroid | 11/50 | |
| Gavage/ 2,3,7,8-TCDD | rats/ Osborne-Mendel | F | 0.0 µg/kg/week | 104 weeks | 105 weeks | corn oil-acetone (9:1) | neoplastic nodule or hepatocellular carcinoma of the liver | 5/75 | |
| | | | 0.1 µg/kg/week | 104 weeks | 107 weeks | corn oil-acetone (9:1) | neoplastic nodule or hepatocellular carcinoma of the liver | 1/49 | |
| | | | 0.05 µg/kg/week | 104 weeks | 107 weeks | corn oil-acetone (9:1) | neoplastic nodule or hepatocellular carcinoma of the liver | 3/50 | |
| | | | 0.5 µg/kg/week | 104 weeks | 107 weeks | corn oil-acetone (9:1) | neoplastic nodule or hepatocellular carcinoma of the liver | 14/49 | |
| Gavage/ 2,3,7,8-TCDD | mice/B6C3F1 | M | 0.0 µg/kg/week | 104 weeks | 105 weeks | corn oil-acetone (9:1) | hepatocellular carcinoma | 8/73 | NTP, 1980a |
| | | | 0.1 µg/kg/week | 104 weeks | 107 weeks | corn oil-acetone (9:1) | hepatocellular carcinoma | 9/49 | |

TABLE 11-24 (cont.)

| Exposure Route/ Compound | Species/Strain | Sex | Dose or Exposure | Duration of Treatment | Duration of Study | Vehicle | Tumor Type | Tumor Incidence | Reference |
|---|---|---|---|---|---|---|---|---|---|
| Gavage/ 2,3,7,8-TCDD (cont.) | mice/B6C3F1 | | 0.05 μg/kg/week | 104 weeks | 107 weeks | corn oil-acetone (9:1) | hepatocellular carcinoma | 8/49 | NTP, 1980a |
| | | | 0.5 μg/kg/week | 104 weeks | 107 weeks | corn oil-acetone (9:1) | hepatocellular carcinoma | 17/50 | |
| Gavage/ 2,3,7,8-TCDD | mice/B6C3F1 | F | 0.0 μg/kg/week | 104 weeks | 105 weeks | corn oil-acetone (9:1) | hepatocellular carcinoma,<br>follicular-cell adenomas of the thyroid | 1/73<br>0/69 | NTP, 1980a |
| | | | 0.04 μg/kg/week | 104 weeks | 107 weeks | corn oil-acetone (9:1) | hepatocellular carcinoma,<br>follicular-cell adenomas of the thyroid | 2/50<br>3/50 | |
| | | | 0.2 μg/kg/week | 104 weeks | 107 weeks | corn oil-acetone (9:1) | hepatocellular carcinoma,<br>follicular-cell adenomas of the thyroid | 2/48<br>1/47 | |
| | | | 2.0 μg/kg/week | 104 weeks | 107 weeks | corn oil-acetone (9:1) | hepatocellular carcinoma,<br>follicular-cell adenomas of the thyroid | 6/47<br>5/46 | |
| Oral/ 2,3,7,8-TCDD | rat/ Sprague-Dawley | M | 0.0 ppb | 78 weeks | 95 weeks | in diet | all tumors | 0/10 | Van Miller et al., 1977a |
| | | | 0.001 ppb | 78 weeks | 95 weeks | in diet | all tumors | 0/10 | |
| | | | 0.005 ppb | 78 weeks | 95 weeks | in diet | all tumors | 5/10 | |
| | | | 0.05 ppb | 78 weeks | 95 weeks | in diet | all tumors | 3/10 | |
| | | | 0.5 ppb | 78 weeks | 95 weeks | in diet | all tumors | 4/10 | |
| | | | 1.0 ppb | 78 weeks | 95 weeks | in diet | all tumors | 4/10 | |
| | | | 5.0 ppb | 78 weeks | 95 weeks | in diet | all tumors | 7/10 | |

## TABLE 11-24 (cont.)

| Exposure Route/ Compound | Species/Strain | Sex | Dose or Exposure | Duration of Treatment | Duration of Study | Vehicle | Tumor Type | Tumor Incidence | Reference |
|---|---|---|---|---|---|---|---|---|---|
| Oral/ 2,3,7,8-TCDD | rat/ Sprague-Dawley | M | 0.0 μg/kg/day | 105 weeks | 105 weeks | in diet | squamous cell carcinoma of the hard palate,<br>squamous cell carcinoma of the tongue,<br>adenoma of the adrenal cortex | 0/85<br>0/85<br>0/85 | Kociba et al., 1978a |
| | | | 0.001 μg/kg/day | 105 weeks | 105 weeks | in diet | squamous cell carcinoma of the hard palate,<br>squamous cell carcinoma of the tongue,<br>adenoma of the adrenal cortex | 0/50<br>1/50<br>0/50 | |
| Oral/ 2,3,7,8-TCDD | rat/ Sprague-Dawley | M | 0.01 μg/kg/day | 105 weeks | 105 weeks | in diet | squamous cell carcinoma of the hard palate,<br>squamous cell carcinoma of the tongue,<br>adenoma of the adrenal cortex | 0/50<br>1/50<br>2/50 | Kociba et al., 1978a |
| | | | 0.1 μg/kg/day | 105 weeks | 105 weeks | in diet | squamous cell carcinoma of the hard palate,<br>squamous cell carcinoma of the tongue,<br>adenoma of the adrenal cortex | 4/50<br>3/50<br>5/50 | |
| Oral/ 2,3,7,8-TCDD | rat/ Sprague-Dawley | F | 0.0 μg/kg/day | 105 weeks | 105 weeks | in diet | hepatocellular carcinoma,<br>squamous cell carcinoma of the tongue,<br>squamous cell carcinoma of the lung | 0/86<br>0/86<br>0/86 | Kociba et al., 1978a |
| | | | 0.001 μg/kg/day | 105 weeks | 105 weeks | in diet | hepatocellular carcinoma,<br>squamous cell carcinoma of the tongue,<br>squamous cell carcinoma of the lung | 0/50<br>0/50<br>0/50 | |
| | | | 0.01 μg/kg/day | 105 weeks | 105 weeks | in diet | hepatocellular carcinoma,<br>squamous cell carcinoma of the tongue,<br>squamous cell carcinoma of the lung | 2/50<br>1/50<br>0/50 | |

TABLE 11-24 (cont.)

| Exposure Route/ Compound | Species/Strain | Sex | Dose or Exposure | Duration of Treatment | Duration of Study | Vehicle | Tumor Type | Tumor Incidence | Reference |
|---|---|---|---|---|---|---|---|---|---|
| Oral/ 2,3,7,8-TCDD | rat/ Sprague-Dawley | F | 0.1 μg/kg/day | 105 weeks | 105 weeks | in diet | hepatocellular carcinoma,<br>squamous cell carcinoma of the tongue,<br>squamous cell carcinoma of the lung | 11/49<br>4/49<br>7/49 | Kociba et al., 1978a |
| Gavage/ 2,3,7,8-TCDD | mice/Swiss/ H/Riop | M | 0.0 μg/kg/week | 365 days | 588 days | sunflower oil | liver tumors | 7/38 | Toth et al., 1979 |
| | | | 0.007 μg/kg/week | 365 days | 649 days | sunflower oil | liver tumors | 13/44 | |
| | | | 0.7 μg/kg/week | 365 days | 633 days | sunflower oil | liver tumors | 21/44 | |
| | | | 7.0 μg/kg/week | 365 days | 424 days | sunflower oil | liver tumors | 13/43 | |
| Oral/ 2,3,7,8-TCDD | mice/ Peramyscus pollenotus | M&F | 0.0012 μg/kg/day | NA | NA | contaminated soil | liver | 0/15 | Cockerham et al., 1980 |
| | | | 0.0 μg/kg/day | NA | NA | contaminated soil | liver | 0/15 | |
| Gavage/HxCDD | rats/ Osborne-Mendel | M | 0.0 μg/kg/week (vehicle control) | 104 weeks | 105 weeks | corn oil-acetone (9:1) | liver neoplastic nodules or hepatocellular carcinoma | 0/74 | NTP, 1980d |
| Gavage/HxCDD | rats/ Osborne-Mendel | M | 1.25 μg/kg/week | 104 weeks | 106 weeks | corn oil-acetone (9:1) | liver neoplastic nodules or hepatocellular carcinoma | 0/49 | NTP, 1980d |
| | | | 2.5 μg/kg/week | 104 weeks | 107 weeks | corn oil-acetone | liver neoplastic nodules or hepatocellular carcinoma | 1/50 | |
| | | | 5.0 μg/kg/week | 104 weeks | 107 weeks | corn oil-acetone | liver neoplastic nodules or hepatocellular carcinoma | 4/48 | |

## TABLE 11-24 (cont.)

| Exposure Route/ Compound | Species/Strain | Sex | Dose or Exposure | Duration of Treatment | Duration of Study | Vehicle | Tumor Type | Tumor Incidence | Reference |
|---|---|---|---|---|---|---|---|---|---|
| Gavage/HxCDD | rats/ Osborne-Mendel | F | 0.0 µg/kg/week | 104 weeks | 105 weeks | corn oil-acetone (9:1) | liver neoplastic nodules or hepatocellular carcinoma | 5/75 | NTP, 1980d |
| | | | 1.25 µg/kg/week | 104 weeks | 107 weeks | corn oil-acetone (9:1) | liver neoplastic nodules or hepatocellular carcinoma | 10/50 | |
| | | | 2.5 µg/kg/week | 104 weeks | 107 weeks | corn oil-acetone (9:1) | liver neoplastic nodules or hepatocellular carcinoma | 12/50 | |
| | | | 5.0 µg/kg/week | 104 weeks | 107 weeks | corn oil-acetone (9:1) | liver neoplastic nodules or hepatocellular carcinoma | 30/50 | |
| Gavage/HxCDD | mice/B6C3F1 | M | 0.0 µg/kg/week | 104 weeks | 105 weeks | corn oil-acetone (9:1) | hepatocellular adenomas or carcinomas | 15/73 | NTP, 1980d |
| | | | 1.25 µg/kg/week | 104 weeks | 108 weeks | corn oil-acetone (9:1) | hepatocellular adenomas or carcinomas | 14/50 | |
| | | | 2.5 µg/kg/week | 104 weeks | 107 weeks | corn oil-acetone (9:1) | hepatocellular adenomas or carcinomas | 14/49 | |
| | | | 5.0 µg/kg/week | 104 weeks | 108 weeks | corn oil-acetone (9:1) | hepatocellular adenomas or carcinomas | 24/48 | |
| Gavage/HxCDD | mice/B6C3F1 | F | 0.0 µg/kg/week | 104 weeks | 106 weeks | corn oil-acetone (9:1) | hepatocellular adenomas or carcinomas | 3/73 | NTP, 1980d |
| | | | 2.5 µg/kg/week | 104 weeks | 108 weeks | corn oil-acetone (9:1) | hepatocellular adenomas or carcinomas | 4/48 | |
| | | | 5.0 µg/kg/week | 104 weeks | 108 weeks | corn oil-acetone (9:1) | hepatocellular adenomas or carcinomas | 6/47 | |
| | | | 10.0 µg/kg/week | 104 weeks | 107 weeks | corn oil-acetone (9:1) | hepatocellular adenomas or carcinomas | 10/47 | |

NA = Not available

intake of 2,3,7,8-TCDD from the diet was 0.0, 0.001, 0.01 and 0.1 μg/kg/day. In the high-dose group, both male and female animals had significant increases in site-specific tumors. The target organs and tumor types in male animals were squamous cell carcinomas of the tongue, squamous cell carcinomas of the hard palate and nasal turbinates, and adenomas of the adrenal cortex; in female animals the target organs and tumor types were hepatocellular carcinomas, squamous cell carcinomas of the tongue and nasal turbinates, and squamous cell carcinomas of the lung. The data demonstrate that dietary exposure to 2,3,7,8-TCDD at levels that produce a daily dose of 0.1 μg/kg results in increased tumor incidences in both male and female rats.

Under the National Toxicology Program, 2,3,7,8-TCDD was tested for carcinogenicity in rats following administration by gavage (NTP, 1980a). Both male and female animals were exposed to weekly doses of 0.0, 0.01, 0.05 and 5 μg/kg bw. The only tumors that appeared to be treatment-related were follicular cell adenomas or carcinomas of the thyroid in male animals, and neoplastic nodules or hepatocellular carcinomas of the liver in female animals. The incidence of these tumors was significantly greater than control in the high-dose groups, and the incidence of both tumors showed a positive dose-related trend. Under the conditions of this assay, 2,3,7,8-TCDD was concluded to be carcinogenic in both male and female rats.

Further studies in mice exposed by gavage have provided support for the carcinogenicity of 2,3,7,8-TCDD. Toth et al. (1979) exposed male mice to 2,3,7,8-TCDD at doses of 0.0, 0.007, 0.7 and 7.0 μg/kg/week in a study to determine whether 2,4,5-TCPE, its contaminant 2,3,7,8-TCDD or both were carcinogens. At the 0.7 μg/kg/week level there was a significantly increased incidence of liver tumors. Liver tumors were not significantly increased in the high-dose group; however, early mortality in this group may have precluded observing late-developing tumors. Similar increased incidences of liver tumors were observed in the NTP (1980a) study in the high-dose male mice exposed to 0.5 μg/kg/week and in the high-dose female mice exposed to 2 μg/kg/week of 2,3,7,8-TCDD by gavage. Female mice also had an increased incidence of follicular-cell adenomas of the thyroid. In both studies, 2,3,7,8-TCDD was carcinogenic to mice, with effective doses ranging between 0.5 and 2 μg/kg/day, depending on sex and the individual study.

The mouse skin two-stage tumorigenicity model has also been used to test the carcinogenic potential of 2,3,7,8-TCDD. Following long-term dermal application 3 times/week of 2,3,7,8-TCDD at levels of 0.01 and 0.005 μg/application to male and female mice, respectively, there was an increased incidence of skin tumors only in female mice (NTP, 1980b). Along with the indication that 2,3,7,8-TCDD was a complete carcinogen in this system, DiGiovanni et al. (1977) reported that 2,3,7,8-TCDD was also a tumor initiator in mouse skin. The ability of 2,3,7,8-TCDD to initiate tumors, however, has yet to be confirmed since appropriate vehicle and promotion-only control groups were not included. Attempts to demonstrate tumor-promoting activity with 2,3,7,8-TCDD on mouse skin have produced negative results in some assays (NTP, 1980b; Berry et al., 1978, 1979); however, Poland et al. (1982) reported that 2,3,7,8-TCDD was a tumor promoter when tested on the skin of mice homozygous for the "hairless" trait, but not in mice heterozygous for this recessive trait. Pitot et al. (1980) also reported that 2,3,7,8-TCDD was a promoter for DEN-initiated hepatocarcinogenesis in rats following parenteral administration of the compounds. On mouse skin, 2,3,7,8-TCDD was a complete carcinogen and possibly a tumor initiator, while no tumor-promoting activity could be attributed to 2,3,7,8-TCDD in the assays. In rat liver initiated with DEN, 2,3,7,8-TCDD was a tumor promoter.

In studies of the interaction of 2,3,7,8-TCDD with other chemical carcinogens, Kouri et al. (1978) reported that 2,3,7,8-TCDD was a cocarcinogen with 3-MC when administered by subcutaneous injection. In the mouse skin bioassay, initiation with simultaneous administration of 2,3,7,8-TCDD and DMBA, however, did not affect tumor yield (DiGiovanni et al., 1977). Similarly, no effect was observed when 2,3,7,8-TCDD was administered either immediately before (5 minutes) or 1 day after DMBA initiation (Berry et al., 1979; DiGiovanni et al., 1977, 1979b; Cohen et al., 1979). When treatment with 2,3,7,8-TCDD occurred 1-10 days before DMBA initiation, 2,3,7,8-TCDD demonstrated a potent anticarcinogenic action. Although 1-5 days prior exposure to 2,3,7,8-TCDD inhibited tumor initiation by BaP, 3-MC and BaP-diol-epoxide, the tumor initiating ability of the latter compound was also inhibited when 2,3,7,8-TCDD exposure occurred either 5 minutes before or 1 day after initiation (DiGiovanni et al., 1980). The increased AHH activity resulting from 2,3,7,8-TCDD exposure may account for the anticarcinogenic activity by altering the metabolism of the initiating compound; however, DiGiovanni et al. (1980) suggest that the inhibition of the initiating activity of BaP-diol-epoxide 1 day after initiation indicates that more than one mechanism participates in the anticarcinogenic activity of 2,3,7,8-TCDD.

HxCDD has also been tested for carcinogenicity in rats and mice treated by gavage and by dermal application to mice (NTP, 1980c,d). In these studies, a 1:2 mixture of 1,2,3,6,7,8- and 1,2,3,7,8,9-HxCDD was tested. In the oral study, animals received HxCDD at doses of 0.0, 1.25, 2.5 or 5.0 μg/kg/week, except for female mice, which received 0.0, 2.5, 5.0 and 10.0 μg/kg/week. In both species and either sex only tumors of the liver occurred at a significantly greater incidence than controls. In male rats and male and female mice, the liver tumor incidence was significantly increased over control values only in the high-dose groups, while in female rats the incidence was significantly greater at both the medium- and high-dose levels. In the study of HxCDD carcinogenicity in mouse skin conducted by NTP (1980c), there were no treatment-related tumors in either the carcinogenicity bioassay or the tumor promotion assay using DMBA as an initiator. It was concluded that this mixture of HxCDD was carcinogenic to rats and mice following administration by gavage; however, there was no tumorigenic activity when HxCDD was applied to mouse skin.

No chronic animal bioassays were found in the literature searched on the carcinogenicity of 1,2,3,7,8-PeCDD.

## 11.2. CASE REPORTS AND EPIDEMIOLOGICAL STUDIES*

11.2.1. Case Reports. Observations of an unusual occurrence of relatively rare soft-tissue sarcomas were first made by Hardell (1977). Of some 87 patients seen from 1970-1976 at the Department of Oncology, University Hospital, Umea, Sweden, seven individuals with soft-tissue sarcomas were identified. All seven had had occupational exposure to phenoxy acids 10-20 years earlier. The tumors were 2 leiomyosarcomas, 1 liposarcoma, 1 rhabdomyosarcoma, 1 myxofibrosarcoma and 2 additional sarcomas of which the histopathology was uncertain, but one was probably a neurofibrosarcoma and the other a rhabdomyosarcoma. The clustering of this rare tumor type among these patients prompted the author to suggest that epidemiological studies be done to determine if exposure to phenoxy acids and the impurities they contain are related to the occurrence of soft-tissue sarcomas.

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*Portions of this section were taken from U.S. EPA (1980c).

Zack and Suskind (1980) reported a soft-tissue sarcoma death in a cohort study of workers exposed to 2,3,7,8-TCDD in a trichlorophenol process accident in Nitro, West Virginia. This tumor, a fibrous histiocytoma, was noted by the author as a rare event. This study, referred to as the Nitro study, is discussed later.

Cook et al. (1980) in a cohort mortality study of 61 male employees of a trichlorophenol manufacturing area, who exhibited chloracne following a 1964 exposure incident, noted four deaths by the end of his study period, one of which was due to a fibrosarcoma. The authors did not seem to attribute any special significance to this finding at the time.

Ott et al. (1980) in a cohort mortality study of 204 employees exposed to 2,4,5-T during its manufacture from 1950 to 1971, found no soft-tissue sarcomas among 11 deaths that had occurred by 1976. One of these 11 deaths was due to a malignant neoplasm.

In a review of the studies of Zack and Suskind (1980), Cook (1980), an unpublished study by Zack (in which a liposarcoma was found), a study by Ott et al. (1980) and Honchar and Halperin (1981) noted 3 (2.9%) soft-tissue sarcomas in a total of 105 deaths. Among U.S. males aged 20-84, 0.07% of the deaths were reported as soft tissue sarcomas (ICD 171, 8th Revision, 1975)* indicating an unusual excess of such tumors. This may be an underestimate because of the possibilities that some soft-tissue sarcomas may have been coded to categories other than ICD 171. Individually, none of the reported case studies reported a significant excess of soft-tissue sarcomas.

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*Department of Health, Education, and Welfare. U.S. Public Health Service. National Center for Health Statistics of the United States, 1974. Vol. II. Mortality, Part A.

Cook (1981a) found an additional malignant fibrous histiocytoma after a later review of the medical records from his earlier cohort study. Cook, who was familiar with the three earlier cases, noted that frank chloracne occurred previously in two cases of the four having a diagnosis of malignant fibrous histiocytoma. A third person diagnosed as having a fibrosarcoma worked in a trichlorophenol (TCP) process area contaminated with 2,3,7,8-TCDD. This individual exhibited facial dermatitis but there was no diagnosis of chloracne. The fourth case (diagnosed as a liposarcoma) was an individual who had been employed earlier in a plant producing 2,4,5-T. Cook (1980) noted that although chloracne was not reported, it could not be discounted. He also noted that all four were cigarette smokers and suggested that smokers with chloracne caused by 2,3,7,8-TCDD exposure may be subject to an increased risk of fibrous soft-tissue sarcomas, although no prior reports have shown soft tissue sarcomas associated with cigarette smoking.

Hardell and Eriksson (1981) discounted this hypothesis by citing that only one of Hardell's seven cases exhibited chloracne before the appearance of the soft-tissue saromcas, and that in their subsequent later case control study, they found no difference in smoking habits between his cases and controls.

Moses and Selikoff (1981) reported a fifth soft-tissue sarcoma in a worker employed at the Monsanto Chemical Company at a time when trichlorophenol and 2,4,5-T were being produced. The worker died of a retroperitoneal neurogenic sarcoma (malignant schwanoma) in 1980 at the age of 58. The employee, before his death, in a detailed occupational history said that he believed he was exposed to these chemicals while he was a truck driver, hauler and maintenance worker, but that he did not work in the production of either chemical. He was a nonsmoker and had no history of chloracne.

Johnson et al. (1981) treated a father and son with soft-tissue sarcomas (the 33-year-old son was diagnosed as having a fibrosarcomatous mesothelioma, while the 53-year-old father had a liposarcoma). Both were exposed to halogenated phenol derivatives. The author noted that 2,4-dichlorophenol can be a precursor of 2,4-D and 2,4,5-T. The father had had prolonged exposure before his disease. The son supposedly had a shorter latency, according to the author. In neither case was the follow-up time given.

Sarma and Jacops (1981) reported three cases of thoracic soft-tissue sarcoma in individuals who were presumably exposed to Agent Orange while serving in Vietnam. The diagnoses were fibrous histiocytoma, mediastinal fibrosarcoma, and a pleural/diaphragmatic leiomyosarcoma. All three served in areas where defoliants were used at the time. One was drenched with the material in one spraying.

Bishop and Jones (1981) found two cases of non-Hodgkin's lymphomas of the scalp in a related clinical study of 158 employees of a pentachlorophenol manufacturing plant in Wales. Homologues of 2,3,7,8-TCDD occurred as contaminants at up to 300 ppm at intermediate manufacturing stages and 5 ppm in the final products. Mild, moderate and severe cases of chloracne were seen in many employees, including the two men who subsequently developed lymphomas. Both men worked in processes where exposure to other chemicals occurred, including exposure to aromatic hydrocarbons. The authors reported that only 0.28 tumors of this type could be expected to occur in a group of 158 workers (ICD 200 and 202), although the basis for the computation of expected numbers is not stated.

Olsson and Brandt (1981) noted that of 123 male patients seen at their clinic in Sweden with a recent diagnosis of non-Hodgkin's lymphoma (NHL), 5 had cutaneous lesions as the only clinically detectable manifestation of

NHL. Four of the five were reported to have repeatedly sprayed large areas with phenoxy acid herbicides. In the remaining 118 NHL patients, only seven had a similar occupational exposure to phenoxy acids. The authors reported this to be significant at $p<0.001$. Olsson and Brandt suggested that a relationship exists between cutaneous presentation of NHL and occupational exposure to phenoxy acids, and believed their observations were similar to those of Bishop and Jones (1981).

The total number of workers with these illnesses who were exposed to phenoxy acids and/or chlorophenols is small, but considering the rarity of this cancer, it is unusual that so many cases of soft-tissue sarcomas have occurred. A Lancet editorial (Anonymous, 1982) calls this phenomenon "disturbing."

### 11.2.2. Epidemiologic Studies.

11.2.2.1. SOFT-TISSUE SARCOMAS -- Soft-tissue sarcomas (STS) constitute a collection of heterologous lesions that include both malignant and nonmalignant tumors. Not all of them have their origin in primordial mesenchymal cells. Some exceptions are tumors of peripheral nerves, and neuroectodermal tumors that are classified as STS but are derived from nonmesenchymal cells. Classification, grading and staging of STSs is difficult because of the capacity of such cells to differentiate into many different tissues. Fairly precise histogenetic classification of such tumors is accomplished through consideration of growth patterns and cell morphology and evaluation of intracellular and extracellular products of tumor cells. There are a dozen distinctly different classes of mesenchymal cells that develop into the following six well-defined tissue complexes: fibrous tissue, tendosynovial tissue, adipose tissue, muscle, vessels and bone.

STSs can be induced in any of these tissue types (Hajdu, 1983). The classification of STSs for cause of death coding in the ninth and latest revision of the International Classification of Diseases (ICD, 1975) places STSs into one of several categories. But chiefly, they fall into "malignant neoplasms of connective and other soft-tissue" (ICD 171). Lymphosarcomas, retroperitoneal sarcomas and extra skeletal STSs of the bone are coded elsewhere. In some instances, if site is mentioned, it is coded to the site [i.e., leiomyosarcoma of the stomach (ICD 151.9), neurofibroma of the chest wall (215.4)].

Questions have been raised concerning the appropriateness of lumping together malignant tumors of different sites and tumor types in order to derive risk estimates. It may not be scientifically appropriate to do so because an elevated risk cannot readily be ascribed to a particular site or type as is usual with most carcinogenic chemicals and substances. Unfortunately, with respect to STSs, tallies of deaths from STSs of particular sites and types are not maintained separately by the vital statistics offices because of their rarity; therefore, it is impossible to derive risk estimates for particular types at given sites. Altogether, ~2000 deaths/year can be attributed to STSs in the United States, most of which are coded to ICD category 171 for purposes of developing incidence and mortality rates for this composite cause. Within ICD 171, individual types that may be correlated with exposure cannot be identified.

A separate problem that potentially could arise from assigning STSs to multiple ICD codes is that incidence and death rates from STSs may be underestimated. Furthermore, risk estimates derived from dividing observed cases (or deaths) by expected cases (or deaths) could be biased upward. This could happen when observed STSs classified to ICD codes other than ICD 171 are lumped together in ICD 171 while expected STSs are based upon STSs classifiable to ICD 171 only. Thus, action of this sort, especially with respect to cohort studies of individuals exposed to dioxin-containing herbicides and/or chlorophenols, could lead to risk estimates that may be biased upward by the inclusion of STSs in the observed category for risk estimation that should be coded to categories other than 171.

Prompted by clinical observations over a 7-year period of malignant sarcomas in seven men with previous occupational exposure to phenoxyacetic acid herbicides (Hardell, 1977), researchers at the Department of Oncology, University Hospital, Umea, Sweden, initiated case-control epidemiologic studies to test the hypothesis of an etiologic association (Hardell and Sandstrom, 1979). Cases were defined as male patients with sarcomas of soft connective tissue, such as smooth muscle (leiomyosarcoma) and fat (liposarcoma). The distribution of tumor types in the two studies is shown in Table 11-25. Sarcomas of tissues, such as bone and cartilage, were excluded as cases. According to the authors, these tumors may have a different etiology and there occurred a different age-distribution in patients with these tumors as compared with that of STS (Hardell, 1983).

Two case-control studies were conducted: the first in northern Sweden (referred to below as Study A) and the second in the southern part of the country (Study B). The exposures to the substances of primary interest are shown in Table 11-26. In the north (Study A), occupational exposure to phenoxyacetic acids took place in both forestry and agricultural work. In the south (Study B), these exposures were predominantly agricultural. The phenoxyacetic acids to which exposure occurred consisted predominantly of 2,4,5-T and 2,4-D in both studies. Exposure to 2,4,5-T in the absence of 2,4-D was rarely reported in either study. Exposure to chlorophenols, which

## TABLE 11-25

## Distribution of Tumor Types in Two Case-Controls Studies of Soft-Tissue Sarcoma

| Diagnosis | Tissue of Origin | Percent of Cases | |
|---|---|---|---|
| | | Study A[a] (n=52) | Study B[b] (n=110) |
| Leiomyosarcoma | Smooth muscle | 30 | 23 |
| Fibrous histiocytoma | Subcutaneous connective tissue | 17 | 25 |
| Liposarcoma | Fat tissue | 14 | 6 |
| Neurogenic sarcoma | Nerve tissue | 10 | 4 |
| Angiosarcoma | Blood vessels | 8 | 2 |
| Myxosarcoma | Primitive connective tissue | 6 | 8 |
| Fibrosarcoma | Fibrous tissue | 4 | 8 |
| Other sarcomas | | 11 | 24 |
| Total | | 100 | 100 |

[a] Unpublished information supplied by Hardell to EPA (Hardell and Sandstrom, 1979)

[b] Eriksson et al., 1979, 1981

## TABLE 11-26

## Exposure Frequencies in Two Case-Control Studies of Soft-Tissue Sarcoma

| Substance(s) | Percent Exposed | | | |
|---|---|---|---|---|
| | Study A | | Study B | |
| | Cases (n=52) | Controls (n=206) | Cases (n=110) | Controls (n=219) |
| Phenoxyacetic acids only | 23.1 | 6.3 | 12.7 | 2.3 |
| Chlorophenols only | 11.5 | 2.4 | 10.0 | 3.6 |
| Both | 1.9 | 0.5 | 0 | 0 |
| Total | 36.5 | 9.2 | 22.7 | 5.9 |

*Sources: Study A, Hardell and Sandstrom, 1979; Study B, Eriksson et al., 1979, 1981 contain chlorinated dibenzodioxin impurities (Levin et al., 1976), occurred mostly in sawmill work and paper pulp production. Very few persons reported exposure both to phenoxyacetic acid and chlorophenols in these studies. Of the two predominant phenoxyacetic acids, only 2,4,5-T is known to be contaminated with 2,3,7,8-TCDD. In Study B, a relative risk of 4.9 (90% confidence intervals 1.6-11.1) was found in relation to exposure to phenoxyacetic acid herbicide other than 2,4,5-T (2,4-D, MCPA, mecoprop, dichloroprop).

Relative risks in relation to the three major categories of exposure are shown in Table 11-27.* Studies A and B indicate a risk of developing STSs among workers exposed to phenoxyacetic acids only, chlorophenols only, or phenoxyacetic acids and/or chlorophenols several times higher than among persons not exposed to these chemicals. In each comparison, the relative risk is high and was thus unlikely to have resulted by chance alone.

Since little is known of the etiology of STSs, the consideration of confounding in these studies was largely a hypothetical matter. The authors presented the effects of age, sex, and place of residence as possible confounding factors in the selection of controls.† Because of the high correlation between exposure to the substances of interest and employment in agriculture and forestry, a possible alternative hypothesis could be that some other unknown factor present in these occupations was responsible for the elevated relative risks.

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*In the analyses considering phenoxyacetic acids only and chlorophenols only, persons exposed to the other categories of substances were excluded. In Study A, the three persons exposed to both chlorophenols and phenoxyacetic acids were included in all comparisons.

†Controls were matched individually to cases on the basis of these factors. Unmatched analyses are presented in Table 11-26 for the sake of simplicity. The matched-method relative risks for exposure to phenoxyacetic acids and/or chlorophenols were 6.2 ($p<0.001$) in Study A and 5.1 ($p<0.001$) in Study B.

## TABLE 11-27

### Relative Risks of Soft-Tissue Sarcoma in Relation to Exposure to Phenoxyacetic Acids and Chlorophenols in Two Case-Control Studies[a]

| | Phenoxyacetic Acids Only | | Chlorophenols Only | | Phenoxyacetic Acids and/or Chlorophenols | |
|---|---|---|---|---|---|---|
| | Study A | Study B | Study A | Study B | Study A | Study B |
| Relative risk[b] | 5.3 | 6.8 | 6.6 | 3.3 | 5.7 | 4.7 |
| 90% Confidence interval[c] | 2.7-10.2 | 3.1-14.9 | 2.8-15.6 | 1.6-7.0 | 3.2-10.2 | 2.7-8.3 |
| Significance level[d] | <0.001 | <0.001 | <0.001 | <0.005 | <0.001 | <0.001 |

[a] Source: Study A, Hardell and Sandstrom, 1979; Study B, Eriksson et al., 1979, 1981

[b] Unmatched odds ratio

[c] Test-based method of Miettinen, 1976

[d] Chi square statistic, no continuity correction, one-tailed test

To test this hypothesis, it is possible to calculate the relative risk in relation to the phenoxyacetic acid exposure in Study B, restricting the analysis to workers within agriculture and forestry. The result is a relative risk of 6.1 (90% confidence interval 2.4-15.4). This finding suggests that a confounding risk factor for STS distributed throughout agriculture and forestry work was not responsible for the overall increase in risk found in relation to phenoxyacetic acid exposure.

Because exposure histories were obtained by means of questionnaires and interviews, the major potential source of bias in these studies stems from the need to rely upon the personal recollection of cases and controls for exposure histories. The published papers indicate that the researchers paid a great deal of attention to this potential problem and specific efforts were made to avoid it during the conduct of the study.

In addition, the relative risk calculated by considering the agriculture and forestry workers who did not report exposure to phenoxyacetic acids or chlorophenols and comparing them with unexposed persons in other occupations was 0.9 (90% confidence interval 0.3-2.4) in Study B. This suggests that little recall bias was present (Axelson, 1980).

In an update of their earlier study, Eriksson et al. (1981) obtained information on the effects of phenoxy acids in the absence of the impurities -- polychlorinated dibenzodioxins and dibenzofurans. The risk ratio given exposure to phenoxy acids free of polychlorinated dibenzodioxins and dibenzofurans equaled 4.2 based upon 7 of 14 respondents who indicated exposure to phenoxy acid herbicides. When consideration was given to persons exposed only to phenoxy acids that contain such impurities, the relative risk was 17.0. A description of the basis for the determination of exposure or nonexposure to dioxins is not well presented in this study.

The author concluded that exposure to phenoxy acids and chlorophenols "might constitute a risk factor in the development of soft-tissue sarcomas." This risk relates not only to 2,4,5-trichlorophenoxy acids containing dioxin impurities but to other phenoxy acids as well. Some doubt was raised concerning the possible misclassification of individuals who were exposed to phenoxy acids free of polychlorinated dibenzodioxins [i.e., in particular, "dichloroprop" in the Eriksson et al. (1981) study]. In a recent communication from Hardell (1983), Eriksson recalculated his risk estimates after reclassifying his dichloroprop-exposed cases and controls into the category of probable exposure to phenoxy acids contaminated with polychlorinated dibenzodioxins and removing them from the nonexposed category. His new estimates were 4.0 based upon 5 of 8 respondents who were exposed to phenoxy acids allegedly free of contamination and 10.9 for those exposed to contaminated phenoxy acid. The first estimate was of only borderline significance utilizing the Mietinen test based statistic, thus, weakening any finding that the risk of STS extends to phenoxy acids free of dioxin.

In a cohort mortality investigation Cook et al. (1980) studied 61 males involved in a 1964 exposure incident who had absorbed 2,3,7,8-TCDD through the skin and developed chloracne. The skin lesions characterizing chloracne ranged from a few comedones on the back of one employee (predating his entry into the process area where exposure could occur) to severe cysts and comedones over the faces, scalps, ears, necks and backs of the remaining employees of the group. Since the main route of exposure was not through the respiratory tract, no measurements of dioxin in the air were provided by the author. On the other hand, the author divided the cohort of 61 males into potentially "high" vs. "low" exposure by place of work based upon dermal exposure, although not stated. Vital status was traced from the data of the incident through 1978. Altogether only 4 deaths were observed by the end of the follow-up, vs. 7.8 expected. Of these, 3 were cancer deaths vs. 1.6 expected. The remaining death was hypersensitive heart disease vs. 3.8 expected. The histopathologic causes of death of the three cancer victims were 1) fibrosarcoma, 2) glioma with metastases, and 3) adenocarcinoma. The authors report that all three victims smoked a minimum of one pack of cigarettes a day for "many years." Not enough information is provided by the authors to conclude that any of these four deaths were smoking related. Site of tumor is not mentioned in the cancer deaths.

Cancer mortality is slightly elevated in this cohort. The study has low sensitivity and lacks a sufficient latent period. This increased mortality was not attributable to any particular cause and no deaths were attributable to liver cancer. Additionally, the authors state that only one of the cancer deaths possessed "documented" evidence of chloracne, although this appears to be at variance with the definition of the cohort, which was reported by the authors to consist of males who reported to the medical department with skin conditions subsequently "diagnosed as chloracne." The authors concluded that the latency period was sufficient to "allow the identification of a potent human carcinogen," since it "exceeded 14 years." Orris (1981) noted that in the Hardell and Sandstrom (1979) study the authors stated that the latent period for soft-tissue tumors may be as long as 27 years and for many, over 14 years. In any case, Hueper and Conway (1964) noted that the latent period for the chemical induction of solid malignant tumors in man exceeds 15 years and is probably <30 years.

Smith et al. (1982b) conducted an initial case-control study of 102 males identified from the New Zealand Cancer Registry as having STSs (ICD 171) between 1976 and 1980. For each case, three controls each with another form of cancer were matched by age and year of registration. The selection of cancer controls from the same registry was done to eliminate recall bias and/or interviewer bias. The distribution of histological types in the cases is given in Table 11-28. An interview to elicit occupational history information was accomplished by telephone either with the next of kin to the patient or the patient himself if he was well enough, although the information was not used in this preliminary analysis.

Comparisons between cases and controls were accomplished by use of occupational groupings according to the Standard Classification System of New Zealand focusing on those occupational groups with a potential for exposure to phenoxy herbicides and chlorophenols. Expected cases for each major occupational classification were derived based upon the occupational distribution of the controls. The authors found no unusual excess of cases of STS in any major occupational category. In agriculture, forestry and fishing, 14 cases were observed vs. 14.0 expected. In laborers, production and transport workers, 35 cases were observed vs. 37.0 expected. A further breakdown of these two broad categories into finer subcategories within the major occupational categories revealed no significant excesses. The study, however, is not useful in assessing the risk of STS from exposure to phenoxy acids and/or chlorophenols for several reasons. First, as was pointed out by the authors but subsequently dismissed by them as having not much of an influence, is the possibility that movement from one major occupational category to another over the time period involved for latent conditions to

## TABLE 11-28

### Distribution of Histological Types of Soft-Tissue Sarcomas*

| Cell Type | Number of Cases | Percent |
|---|---|---|
| Fibrosarcoma | 25 | 24 |
| Liposarcoma | 20 | 20 |
| Rhabdomyosarcoma | 9 | 9 |
| Leiomyosarcoma | 7 | 7 |
| Malignant Histiocytoma | 6 | 6 |
| Other | 22 | 21 |
| Unspecified | 13 | 13 |
| Total | 102 | 100 |

*Source: Smith et al., 1982b

manifest themselves could introduce a negative bias into any estimates of relative risks. The latency for STS was suggested to be a minimum of 15 years (Hueper and Conway, 1964).

The finding of no switching from one occupational category to another that was noted in the "first 20 interviews" in which a change could be noted is not necessarily indicative of fidelity to the same job over long periods in all 408 cases and controls. Information identifying a change may be lacking in those cases and controls if in fact one did occur possibly because of several reasons, for example, separation of the earlier work history from the latter and purging of earlier employment records. Besides the "first 20 interviews" where a change could be noted is not necessarily representative of the entire cohort in any case.

Furthermore, the authors do not know absolutely that any of their cases and controls were exposed to phenoxy acids or chlorophenols or to both since apparently no effort was made to confirm "potential" exposures. Only differences in occupational classification were noted where "potentially" cases or controls could have had exposure to the dioxin-containing herbicides. It was pointed out that the risk estimates noted do not "preclude" the possibility that an association may be found in this study when the cases and controls (or surviving kin) are interviewed for chemical spraying at a later time. The authors tnemselves concluded that the preliminary study results "should not be taken as substantial evidence against the hypothesis that phenoxy herbicides and chlorophenols may cause human cancer."

The distribution of tumor types differed considerably from the Hardell and Eriksson study to the Smith study. Leiomyosarcomas, malignant histocytomas, neurogenic sarcomas and myxosarcoma seem to predominate in the Hardell and Eriksson study, whereas fibrosarcomas and liposarcomas appear prominently in the Smith study. More attention should be devoted to the study of the distributions of STS types in registry data everywhere in order to determine if such variations in the reporting of STS types are random occurrences. It is possible that the cancer effect of exposure to phenoxy herbicides may be narrowed to just certain types of STSs, the predominant ones in the Swedish studies.

In a later study of STSs, Smith et al. (1983a) conducted a case-control study of STSs in males that were reported to the New Zealand Cancer Registry by Public Hospitals between 1976 and 1980. The author matched one cancer control randomly chosen from the registry with each case, initially starting with 112 of each. Controls were matched for year of registration and by date of birth ± 2 years. Inquiries were made by the authors with the hospital consultant, family doctor, and finally the next-of-kin or patient if alive. Telephone interviews were conducted by only one interviewer, who had no knowledge of the patient's cancer history, and were completed on 80 cases and 92 controls. Because some 32 potential cases (14 ineligible) and 20 controls were excluded or lost from the study for various reasons, it raises a question whether control of confounding by age and year of registration was maintained in the final group of 172 cases and control included in the analysis. Presumably the corresponding "matched" case or control to each of the 52 lost members of the total study group were not excluded. However, since the span of registration was only 5 years, not much age confounding could occur.

Patients were classified as having had potential exposure to phenoxyacetic acids if they had definite, probable or possible exposure to phenoxyacetic acid through spraying or hand contact. The actual chemical was identified only in some instances. The authors concluded in all remaining situations that if the member sprayed "gorse" and/or "blackberries" this was tantamount to potential exposure to phenoxyacetic acid. Smith (1983) calculated elevated but nonsignificant relative risks of exposure to phenoxyacetic acid ranging from 1.3 in those individuals who were "probably exposed" for a minimum of 5 days not in the previous 10 years before cancer registration to 1.6 in individuals "probably exposed" for a minimum of 1 day not in the previous 5 years before cancer registration. When risk ratios were calculated after stratifying by year of birth and whether or not the patient or a relative was interviewed, the rates increased to 1.7 (from 1.6) in the latter and 1.4 (from 1.3) in the former calculation, although still nonsignificant. If the numbers would allow, it would be of interest to repeat the above calculations excluding only those with potential exposure occurring only within the 15-year period just before cancer registration. The small numbers that remain following the 15-year lapse probably precludes such an analysis. Furthermore, the categories of exposure "probably or definitely" exposed for $\geq$1 day or even 5 days raises a question whether any of the cases or controls could really be said to have ever come in contact with enough phenoxyacetic acid to justify such a designation. It could be that, in fact, potentially exposed individuals in New Zealand have had little or no contact with the herbicide.

The authors did conclude that the finding of a relative risk of 1.7 in individuals with $\geq$1 day exposure not in the last 5 years cannot be entirely discounted. But then the authors stated that if length of exposure was $\geq$5 days prior to 10 years before cancer registration, they would expect an increase, and since they do not see an increase, there is no evidence of a "real causal link." One might ask whether this is a suitable criterion for providing evidence of a causal association. Perhaps a more valid group for study would be one where the potential exposure was considerably longer than "5 days" and >15 years before initial cancer registration. As kind of a subtle justification for the finding of no significant risk in workers exposed in phenoxy acids, the author alluded to the fact that there were 500 full-time workers registered in New Zealand who did full time ground spraying and altogether some 2000 workers who were at some time professionally involved in phenoxyacetic acid herbicide spraying from the air or ground with exposure "very much greater" than that of patients in this study. This kind of argument has appeal if these workers could be shown to have had their exposure sufficiently far in the past that latency considerations could be adequately addressed. However, the real question again remains; how much real exposure did those patients in the study really have 10-15 years earlier, and in what numbers. The author remarked that it was surprising that he found no STS victims who had ever worked full-time in phenoxyacetic acid herbicide spraying. Perhaps they have not yet been observed for a long enough period. The time interval of 10 years and/or 5 years from exposure to registration may not have been long enough to allow latent effects to become evident. However, as was pointed out by the author, the findings do not support the hypothesis that exposure to phenoxyacetic acid herbicides causes STS. But neither do they support a negative finding without better documentation regarding actual exposure and time of actual exposure. Smith (1983), however, noted that his documentation of exposure to 2,4,5-T (and 2,4-D) was at least as good as that in the Hardell and Sandstrom (1979) study, and that although Hardell and Sandstrom (1979) noted higher relative risks of <30 days exposure, Smith (1983) did not. Hence the paradox. Smith (1983) admitted the possibility that 2,3,7,8-TCDD contaminations might be lower in New Zealand as opposed to 2,3,7,8-TCDD contamination in the Swedish studies, although there is no evidence for it.

He still maintains that his study showed that exposure to phenoxyacetic acids may not be associated with STS.

Pazderova-Vejlupkova et al. (1981) studied 80 workers involved in the production of 2,4,5-sodium trichlorophenoxyacetate and butylester of trichlorophenoxyacetic acid who subsequently became ill from exposure to 2,3,7,8-TCDD during the period 1965-1968. Only 55 members of this group were followed for 10 years. The remaining 25 either refused participation or moved leaving no forwarding address. Most patients developed chloracne while 11 developed porphyria cutanea tarda. Chief chemical signs were metabolic disturbances, pathologically elevated lipids with abnormalities in the lipoprotein spectrum, and "pathological" changes in glucose tolerance. Other symptoms noted were biochemical deviations consistent with "a mild liver lesion," light steatosis, periportal fibrosis or activation of Kupffer cells, or nervous system focal damage (peripheral neuron lesion in lower extremities). Altogether six patients were reported to be deceased during this 10-year period, 2 from bronchogenic carcinoma, 1 from cirrhosis, 1 atherosclerosis precipue cerebi and 2 in auto accidents. No STSs or lymphomas were found. Since there was no comparison population with which to estimate relative risk for cancer, the study must be classified at best as clinical with respect to cancer. The 6 deaths (of 55) that occurred during the 10-year observation period cannot be construed to be associated with exposure to the 2,4,5-T. Because of the small number of cases and the short follow-up period, nothing can be said concerning the association of exposure with cancer, especially specific types of cancer such as STS or non-Hodgkin's lymphoma.

Riihimaki et al. (1982, 1983) studied a cohort of 1926 herbicide applicators formed in 1972 from personnel records of four Finnish employers (e.g., the Forestry Authority, Highway Authority, State Railways and a state-owned electric power company). Chlorinated phenoxyacids had been used since the 1950s in Finland for spraying. They constituted 2:1 mixtures of emulsified esters of 2,4-D and 2,4,5-T dissolved in water. Analyses from old herbicide formulations dating back to the 1960s revealed that these mixtures contained 0.1-0.9 mg/kg of 2,3,7,8-TCDD.

This cohort of male workers was exposed a minimum of 2 weeks during at least one growing season from 1955-1971. Follow-up continued 9 years through 1980 for mortality but only until 1978 for morbidity. Fifteen individuals could not be traced by 1980. Expected deaths were generated based upon cause- and age-specific national Finnish death rates for 1975. Expected cases were similarly calculated based upon national incidence rates of 1975.

By 1980, 144 deaths had occurred vs. 184.0 expected, a deficit of 22% in observed mortality. Only 26 cancer deaths had occurred vs. 36.5 expected, a 29% deficit. The authors separated out "natural" deaths from the total. The observed residual deaths equaled 39 while the expected deaths equaled 28.7. This excess was of borderline significance. The authors also considered 10-year and 15-year latent periods. Even after 15 years, the deficit of deaths continued to manifest itself both in categories of all causes and total cancers; 35 observed vs. 53.6 expected and 5 observed vs. 11.3 expected, respectively. Similarly, the 7-year follow-up of cancer morbidity revealed 26 cases of cancer vs. 37.2 expected. After a 10-year latent period, 16 cancer cases were observed vs. 20.1 expected. None of the 26 cancer deaths or 26 cancer cases were of the STS or lymphoma type. (However, only 0.1 STS and 0.5 lymphomas were expected.) In no instance was cancer of any site significantly elevated.

The authors noted that this unusual deficit of mortality and morbidity of between 70 and 82% (even after 15 years from initial exposure) was probably a consequence of the "healthy worker effect" in that only able-bodied and healthy individuals were selected into the industry. The fact that the cohort was assembled in 1972 from records of persons who were exposed as early as 1955 (17 years prior) raises the likelihood that in 1972 a "survivor" population remained (45 deaths before 1972 were eliminated from the cohort) that was relatively healthy. Furthermore, the unusually large number of not "natural" expected and observed deaths (probably accidents and external causes) occurring to this cohort indicate a relatively youthful population was under scrutiny. The leading cause of death to persons under 35 years is from accidents, based on national vital statistics.

The authors correctly noted that, because of limitations in the study material, only powerful carcinogenic effects could be detected. Risk ratios higher than 1.5 for all cancers, 4.0 for lymphomas and 10.0 for STS could be excluded based on this data set from the authors' own calculations. More follow-up is needed in order to provide a stable assessment of the relationship between exposure and cancer. The authors concluded that this study will allow no assessment of STS because "the number of persons having a sufficiently long latency period is too small." It was suggested that more valid conclusions could be made only with the passage of time (Riihimaki et al., 1983).

Recently, the Michigan Department of Public Health (1983b), produced an ecological study of soft and connective tissue cancer mortality rates in Midland and other selected Michigan counties. They found that mortality rates for this cause were 3.8-4.0 times the national average for the periods 1960-1969 and 1970-1978, respectively, for white females in Midland. These estimates are based upon 5 deaths and 7 deaths, respectively, and are listed in Table 11-29. No excess risk was reported among white males, however. The Michigan Department of Health concluded that because of the occurrence of these two successive elevated rates, it is unlikely to be a chance happening. At the same time the age-adjusted male and female cancer mortality rates for Midland were below that of the State of Michigan for the period 1970-1979. Midland County is the home of a major chemical company that produced phenoxyacetic acid herbicides until recently. The authors stated that a detailed review of death certificates, hospital records, residency and occupational histories of the 20 male and female cases revealed no "commonalities" suggesting a "single causative agent," although a majority or their spouses had worked at this chemical facility. They recommend that a case-control study should be employed to evaluate possible influences, such as lifestyle, occupation or location of residence on the risk of STS.

In a series of reports prepared under the auspices of the U.S. Air Force, Col. William H. Wolfe and his associates just completed the first phase of a study of Air Force personnel involved in the aerial dissemination of TCDD-containing herbicides in the Republic of Vietnam (RVN). During the period of time beginning in 1962 and ending in 1971, ~1278 male Air Force personnel (Ranch Handers) were identified as having been involved in the effort to 1) defoliate vegetation in Vietnam in order to decrease the risk of ambush and 2) destroy enemy crops (Wolfe et al., 1985). Based on an 1984 report of baseline mortality study results (Wolfe et al., 1984), the cohort involved in the mortality study was smaller at 1256 because of the

## TABLE 11-29

### Midland County Soft and Connective Tissue Cancer Deaths 1960-1981*

| Identification | | | Type, Site and Progression of Malignancy | | | |
|---|---|---|---|---|---|---|
| Year of Death | Sex | Age | Type | Primary Site | Metastases | Month and Year Diagnosed |
| 1961 | F | 24 | Hemangiosarcoma | Face | Skull and upper lobe of lung | 5-58 |
| 1963 | F | 75 | Liposarcoma | Right gluteal | Unknown | Unknown |
| 1964 | F | 51 | Leiomyosarcoma | Uterus | Widespread | 11-63 |
| 1968 | F | 37 | Liposarcoma | Spine | Lungs, pelvis | 1-66 |
| 1969 | F | 45 | Fibrosarcoma<br>Leiomyosarcoma | Right thigh<br>Uterus | Lung, liver<br>Adrenal gland and skin | 10-68 |
| 1970 | F | 59 | Kaposi sarcoma | Right leg | Lymph nodes | 8-68 |
| 1970 | F | 56 | Fibrosarcoma<br>Leiomyosarcoma | Right thigh<br>Abdominal wall | Spine<br>Lung | 1960<br>1967 |
| 1974 | F | 1 | Rhabdomyosarcoma | Inguinal area | Unknown | 8-73 |
| 1976 | F | 77 | Liposarcoma | Right thigh | Buttock, lung, rib, lymph nodes | 12-74 |
| 1978 | F | 64 | Leiomyosarcoma | Left knee | Liver, lymph nodes, lung, bone | 7-70 |

## TABLE 11-29 (cont.)

| Identification | | | Type, Site and Progression of Malignancy | | | |
|---|---|---|---|---|---|---|
| Year of Death | Sex | Age | Type | Primary Site | Metastases | Month and Year Diagnosed |
| 1978 | F | 26 | Rhabdomyosarcoma | Rectum | Lung, neck, inguinal region | 6-76 |
| 1978 | F | 88 | Fibrosarcoma | Right cheek | Facial area | 6-78 |
| 1979 | F | 27 | Leiomyosarcoma | Left thigh | Lung | 3-78 |
| 1962 | M | 63 | Rhabdomyosarcoma | Left lower leg | Lung and right outer chest wall | 8-61 |
| 1967 | M | 77 | Mesothelioma | Lung | Lung, peritoneum and diaphragm | 6-67 |
| 1967 | M | 20 | Rhabdomyosarcoma | Pharynx | Periorbital area and liver | 1-67 |
| 1969 | M | 32 | Liposarcoma | Left arm | Perineum and buttock | 6-64 |
| 1971 | M | 76 | Leiomyosarcoma | Small intestine | Liver | 10-69 |
| 1972 | M | 89 | Leiomyosarcoma | Retro-peritonal region | Hepatic system | 7-72 |
| 1976 | M | 53 | Fibrosarcoma | Peritioneum | Lung, liver | 3-75 |

*Source: Adapted from Michigan Department of Public Health, 1983b exclusion of 22 killed in action and was divided into three main occupational categories as follows:

| | | |
|---|---|---|
| 1. | Officers (pilots, navigators and others) | 466 |
| 2. | Enlisted (flight engineers) | 206 |
| 3. | Enlisted (others) | 584 |
| | TOTAL | 1256 |

The authors categorized the Ranch Handers as having had "exposure" to the TCDD-containing herbicides if they were involved in the aerial spraying of the herbicides. They were matched to 6171 cargo mission air crew members and support personnel generally on a 5 to 1 basis according to similarity of training and military background experiences, occupation and race. The comparison population presumably had no exposure to TCDD. In an earlier 1983 report (Lathrop et al., 1983), 50 deaths were identified in the study group versus 250 in the comparison population. Of these 50 deaths, 23 were due to external causes, 4 were malignant neoplasms, 16 were circulatory causes, 5 were digestive disorders and 1 was an endocrine disorder.

In the later December 1984 update, Wolfe et al. (1984) added 4 more deaths to the study population for a total of 54 deaths occurring to Ranch Hands while adding 15 to the 250 that had already occurred in the comparison group through December 31, 1983. Altogether this update produced a total of 6 cancer deaths in the Ranch Hands versus 43 cancer deaths in the comparison population. The greatest cause of death in both Ranch Handers and the comparison population were accidents with 19 and 94, respectively. None of the 6 cancer deaths and 1 of the 43 deaths in the comparison group were STSs. Comparison of overall mortality in the Ranch Handers with other Air Force military personnel was nearly identical (~4.3%). Ranch Hand ground enlisted personnel suffered somewhat greater (although not significant) mortality than did Ranch Hand officers. Comparison of mortality in the Ranch Handers with other groups such as U.S. white males, Department of Defense retired enlisted men, U.S. civil servants, active duty Air Force and West Point officers from the class of 1956, were similar except for Air Force active duty officers who exhibited significantly less mortality. The authors attribute this to higher health qualification standards.

There were few biological markers that might tend to support the assumption that Ranch Hands were exposed to 2,3,7,8-TCDD. In the Banbury report, Lathrop et al. (1984) reported that the dermatologic evaluation revealed no cases of chloracne through clinical diagnosis or bioassay. A questionnaire analysis of acne in Ranch Handers and comparison groups showed no unusually different incidence, severity, duration or distribution of anatomical locations in either group. Lathrop et al. (1984) said in fact that the "historical occurrence of chloracne was highly unlikely in the Ranch Handers".

This study suffers from several deficiencies that limit its usefulness in a determination of human health effects, notably cancer, and especially STS from exposure to 2,3,7,8-TCDD-contaminated phenoxy herbicides. First, it is mainly a study of basically young men who were involved in the Air Force aerial spraying missions. This is evidenced by the exceptionally large number of accidents attributable to members of the cohort. It is the largest single cause of death in these men. Because this is a young group it is unlikely that substantial mortality will occur to the cohort until many more years of follow-up have passed. In fact, even after 15 years following initial exposure <5% of the cohort have died. Since most cancers have a latency of ≥15 years following initial exposure it is not likely that a cancer risk from 2,3,7,8-TCDD, if any, will manifest itself for some time.

Furthermore, the relatively rare STS, which is thought to have an even longer latency period, may not appear as a risk in this cohort until well after the 20th year. Additionally, this cohort exhibits little evidence of actual exposure to the herbicide in question, thus raising the possibility of misclassification. In other small cohort studies (Cook et al., 1980; Ott et al., 1980; Zack and Suskind, 1980) substantial numbers of the study cohorts exhibited evidence of exposure to 2,3,7,8-TCDD as indicated by the presence of chloracne, a clear biological marker. Few of the Ranch Handers exhibited evidence of this condition (Lathrop et al., 1984). In fact, as was suggested by the authors, the historical occurrence of chloracne was considered highly unlikely in the Ranch Hands. Neither do they present convincing evidence of other conditions suggestive of an association with exposure to the dioxin-containing herbicide that cannot be explained by confounders, according to the authors. In fact Ranch Handers, who were heavily populated with officers, pilots, navigators and flight engineers, may not have been as heavily exposed to the phenoxy herbicides as other U.S. military personnel in Southeast Asia. Perhaps Army combat foot soldiers or the non-Ranch Hand personnel who did the spraying on the ground around the military bases would constitute a more appropriate cohort for study. Lathrop et al. (1984) concluded that the absence of any association of "clinical endpoints" with herbicide exposure must be viewed as insufficient evidence supporting a cause-and-effect relationship. But this absence of any "clinical endpoints" might also indicate evidence of a lack of exposure to the phenoxy herbicides in question by the Ranch Handers. This study must be viewed as inadequate in assessing the risk of cancer from exposure to 2,3,7,8-TCDD-containing phenoxy herbicides.

In a separate review of the epidemiological evidence for STS from exposure to 2,4,5-T-containing herbicides, the United Kingdom Ministry of Agriculture, Fisheries and Food (1983) concluded that there was no evidence to recommend altering their earlier conclusion that formulations of phenoxy acid herbicides and related wood preservatives as "presently cleared" are safe and may continue to be used. This report readily discounts the positive studies of Hardell and Eriksson (1979) as being biased, and it makes no reference to the later validity study by Hardell (1981) of his own work utilizing colon cancer controls (see Section 11.2.2.2.). In this report Hardell answered these early criticisms that were reiterated by the British in their report. At the same time, the British report appears to put undue emphasis on nonpositive studies that do not demonstrate a risk, although most of them have methodological limitations (e.g., low power, insufficient latency and inappropriate study methods). In short, the British review appears to be overly optimistic about the safety of 2,4,5-T herbicides.

Fingerhut et al. (1984) recently completed a review of medical and available exposure records of seven U.S. chemical workers that have been diagnosed as having STS and who were reported to have had possible exposure to dioxin. These cases collectively produced a clustering effect of the relatively rare STSs among former employees of a portion of the U.S. chemical industry where exposure to compounds contaminated with 2,3,7,8-TCDD is most likely to have occurred. Fingerhut et al. (1984) reported that a subsequent review of the Armed Forces Institute of Pathology and a review of one of the authors of the Fingerhut paper confirmed the diagnosis of 5 of the 7 U.S. chemical workers as STSs.

In terms of occupational exposure, Fingerhut et al. (1984) proposed a strict definition of exposure as follows: a record must exist somewhere that shows an assignment to either a 2,4,5-T department or to a trichlorophenol department at some time in the past. If such a record did not exist, then the individual would not have been considered to have had a confirmed exposure. Four of the seven who had a confirmed exposure in this manner were also members of cohorts that had been studied previously, while the remaining three could not be confirmed as having been assigned to any 2,4,5-T department or trichlorophenol department. The latter three were not identified as having been part of any earlier study but were case reports of Johnson et al. (1981) and Moses and Selikoff (1981). Individuals who were members of study cohorts of "exposed individuals" might be expected to have better documentation of exposure, based upon employment records, than would cases turning up in a medical practice.

However, Fingerhut et al. (1984) pointed out that of these three cases, one worked 32 years in production, clerical, truck driving and maintenance jobs in a chemical manufacturing site that produced trichlorophenol and 2,4,5-T; the second worked 2.5 years as a production worker in a plant that made 2,4,5-T; and the third was a production and maintenance worker for 29 years at the same facility as the second worker. It would seem that the opportunity for exposure to 2,3,7,8-TCDD containing 2,4,5-T or trichlorophenol must be considered a distinct possibility in the first two cases, especially since both were involved with maintenance for many years.

Johnson et al. (1981) pointed out that the second case could not have satisfied a minimum latency requirement for exposure to TCDD since his 2.5 years as a production worker occurred just before his diagnosis and death.

However, this man's father was employed with this same plant almost as long as his son was alive and it seems plausible that because of this connection the son may have been exposed.

One must have reservations about the usefulness of a classification scheme that relies on documentation of an assignment to a specific area of a plant as proof of exposure to dioxin without real evidence substantiating that exposure (i.e., either biological or physical measurements), while at the same time assignment to all other areas of the same plant is considered insufficient evidence of exposure although nothing is offered to substantiate the presence or lack of exposure to 2,3,7,8-TCDD in either case. In most occupational prospective cohort epidemiologic studies, employment at a plant where the suspect agent is produced or found has been considered sufficient enough to call such a person "exposed" and thus included in a cohort for study. On the other hand, if the Fingerhut et al. (1984) definition were retrospectively applied to the already small occupational cohorts from which the first four STSs came, even two of these relatively rare STSs might probably constitute an excessive risk in the much smaller cohorts circumscribed by their definition. Fingerhut et al. (1984) agreed that an excess risk of STS would remain even with just two confirmed cases, and hence the possibility of a causal relationship between exposure to 2,3,7,8-TCDD and the development of STSs cannot yet be ruled out.

In summary, the associations reported in the two Swedish soft-tissue sarcoma studies are strong enough to make it unlikely that they have resulted entirely from random variation bias or confounding, even though the possibility cannot be excluded. These studies provide a strong suggestion that phenoxyacetic acid herbicides, chlorophenols or their impurities are carcinogenic in humans.

**11.2.2.2. MALIGNANT LYMPHOMAS** -- A separate series of clinical observations at the Department of Oncology in Umea, Sweden (Hardell, 1979), led the researchers to conduct a case-control study of malignant lymphoma in relation to phenoxyacetic acid, chlorophenols, and other organic compounds (Hardell et al., 1980, 1981). Approximately 33% of the cases in this study were patients with Hodgkin's disease; the remainder of the cases were non-Hodgkin's lymphomas.

This study employed essentially the same methods and produced results comparable with those of the STS studies: statistically significant 5-fold to 6-fold relative risks in relation to phenoxyacetic acids and chlorophenols were confirmed. In addition, an elevated relative risk was found in connection with exposure to organic solvents, such as benzene, trichloroethylene, and styrene. In the published report, the methods and results were incompletely documented, especially the possibility of confounding by exposure to the organic solvents.

In the update of the earlier 1980 study, Hardell et al. (1981), utilizing the same basic data source, found that 36.1% of the cases had been exposed to phenoxy herbicides or chlorophenols, while only 9.6% of their controls were so exposed. The estimated relative risk was 6.0 when matching was considered and 5.3 when matching was eliminated. When cases and controls that were exposed to chlorophenols only were excluded, the relative risk of lymphoma from phenoxy acids alone was 4.8 (95% C.I. 2.9-8.1). On the other hand, if exposures to phenoxy acids are excluded and consideration is given to just chlorophenols (which includes combined exposure to phenoxy acids and chlorophenols), then the relative risk equaled 4.3 (95% C.I. 2.7-6.9). The author further subdivided this group into "low-grade" vs. "high-grade" exposures to chlorophenols. A continuous exposure of not more than 1 week or repeated intermittent exposures totaling not more than 1 month was classified as low-grade. The relative risk for high-grade exposure was 8.4 (95% C.I. 4.2-16.9), while that for low-grade exposure equaled 9.2 (95% C.I. 1.6-5.2). If exposure to organic solvents is examined, given that cases and controls exposed to only phenoxy acids and/or chlorophenols were excluded except for combined exposure to organic solvents, it is found that high-grade and low-grade relative risks were 2.8 (95% C.I. 1.6-4.8) and 1.2 (95% C.I. 0.5-2.6), respectively. However, the author noted that exposure to phenoxy acids and high-grade organic solvents (exposure to chlorophenols excluded) produced a relative risk of 11.2 (95% C.I. 3.2-39.7) based upon a few cases and controls with exposure to both. The authors concluded that "exposure to organic solvents, chlorophenols and/or phenoxy acids constitutes a risk factor for malignant lymphoma."

The Hardell et al. (1981) study is still subject to the same methodological criticisms to which the earlier study was subjected. Chief among those is the possibility of observational and/or recall bias creeping into the responses that are elicited from self-administered questionnaires on kind and length of exposure. Secondly, confounding by exposure to potentially carcinogenic organic solvents and other agents could have had an effect on the risk estimate, although Hardell (1981) insists that they did not.

Other research has tentatively suggested that lumberjacks may be at increased risk of lymphoma (Edling and Granstam, 1979). The Nitro study found three deaths from cancers of the lymphatic and hematopoietic system, against only 0.88 expected (p=0.06, one-tailed Poisson test).

The lymphoma case-control study (Hardell et al., 1980, 1981) is consistent with the two STS studies discussed above. On the other hand, the consistency could also reflect an (as yet) unidentified common flaw in all these studies.

The two Swedish case control studies on STSs and a later case control study of malignant lymphoma (Hardell et al., 1981) were subjected to a validity analysis with respect to the assessment of exposure by Hardell and Eriksson (1981). To answer the question raised regarding the recall of occupation in a forestry/agriculture job, secondary to the recall of exposure to phenoxy acids and/or chlorophenols, the cases and controls were divided into three groups: those who worked their entire time since 1950 in an agriculture/forestry job; those who worked some time in an agriculture/forestry job but not exclusively; and the remainder who never worked in a forestry/agriculture job. The study found that the risk ratio was still 8.2 for STS in exclusively agriculture/forestry workers who were exposed to phenoxy acids compared with workers found in other occupations having no apparent exposure to phenoxy acids or chlorophenols. Even when comparing phenoxy acid- and/or chlorophenol-exposed agricultural/forestry workers exclusively with nonexposed agricultural/forestry workers, the risk ratio was still 7.1. This argument seems to answer effectively questions regarding recall of occupation secondary to exposure.

On the other hand, the relative risk remains 5.4 when comparing phenoxy acid and/or chlorophenol exposed workers exclusively in occupations other than agriculture/forestry with nonexposed workers in those same occupations, thus, suggesting the presence of either recall bias or still another occupation with potential exposure to phenoxy acids and/or chlorophenols (Table 11-30).

When woodworkers are separated out (possible exposure to chlorophenols in treatment of wood) the risk ratio becomes 9.7 (Table 11-31). These data suggest the presence of some recall bias.

## TABLE 11-30

## Other Occupations (Minus Forestry/Agriculture)*

| Group | Phenoxy Acids/Chlorophenols | Non-exposed |
|---|---|---|
| Cases | 11 | 68 |
| Referents | 5 | 167 |
| | RR = 5.4 | $X^2$ = 11.01 (P<0.01) |

*Source: Hardell and Erikkson, 1981

RR = Relative risk

## TABLE 11-31

### Other Occupations (Minus Forestry/Agriculture/Woodworkers)*

| Group | Phenoxy Acids/Chlorophenols | Non-exposed |
|---|---|---|
| Cases | 4 | 66 |
| Referents | 1 | 160 |
| | RR = 9.7 | $X^2 = 5.98$ ($P<0.05$) |

*Source: Hardell and Erikkson, 1981

RR = Relative risk

Another focus of the Hardell and Erikkson (1981) study was to determine if observational bias on the part of the investigators could explain the significantly high risk estimates. To answer the question, the study compared the exposure data derived from the interviewee's returned questionnaires only with the combined information from both the phone interviews and questionnaires. The study found no substantial differences in the frequency of reporting exposure.

Still a third consideration of possible bias involves recall of exposure to phenoxy acids and/or chlorophenols because of subject knowledge of having cancer in the cases versus no knowledge of cancer in the referent population. The study chose as a referent group for the 52 STS cases (Hardell and Sandstrom, 1979) and the 169 malignant lymphomas (Hardell et al., 1981) a group of 154 colon cancer cases from the same population source and compared their exposure to phenoxy acids and/or chlorophenols by broad age groupings, and by rural vs. urban residence.

Utilizing a Mantel-Haenszel rate ratio, the study found the risk of exposure to phenoxy acids remaining significantly high at 5.5 and to chlorophenols 5.4 in the STS cases compared with the colon cancer controls. Similarly, with the malignant lymphomas, the identically derived risk ratios remain significantly high at 4.5 with respect to phenoxy acids and/or chlorophenol exposure in the cases, hence, the study concludes, no "substantial observational bias" exists. If it is assumed in this study that recall bias was and is the same as observational bias, then such a conclusion may not be entirely warranted from the comparison. Certainly, it appears that no recall bias existed because of subject "knowledge of having cancer" based on the authors' analysis. But it does not rule out the possibility that recall bias can still be present in their data for other reasons. Hardell et al. (1981) refers to an intense "debate about phenoxy acids and their presumptive risk" in Sweden at the time the colon cancer study was conducted. But, there is no reason to think that colon cancer victims would assume their disease was brought about from exposure to dioxin containing chemicals if no connection was suggested.

It seems plausible that STS and non-Hodgkin's lymphoma patients would either learn at the time of their diagnosis that exposure to dioxin-containing chemicals was the likely cause of this rare type of tumor or quickly learn from other sources, such as the news media, that exposure to herbicides containing dioxin could cause this rare form of cancer whereas colon cancer victims (a rather common form of cancer) would not necessarily be led to believe that exposure to the same dioxin-containing chemicals caused their disease. Hence, it is not difficult to imagine that such unusual victims of cancer could better "remember" exposure to such chemicals than could colon cancer patients.

Therefore, although the Hardell (1981) study may explain any biases introduced from secondary recall of occupation, observational bias introduced from the telephone interviewer and recall bias based on subject knowledge of cancer, it does not adequately answer questions of recall bias introduced through the acquired awareness on the part of the victim of STS or non-Hodgkin's lymphoma that his condition may have been caused by exposure to dioxin-containing herbicides.

11.2.2.3. STOMACH CANCER -- Studies of two of the oldest cohorts of workers known to have been exposed to 2,3,7,8-tcdd containing phenoxyacetic acid herbicides report stomach cancer mortality rates significantly higher than expected. The results in each study were based on small numbers of deaths. In one study (Axelson et al., 1980), 348 Swedish railroad workers with at least 46 days of herbicide exposure between 1955 and 1972 were followed through October 1978. The workers were grouped on the basis of their primary herbicide exposures: those primarily exposed to phenoxyacetic acids (2,4-D and 2,4,5-T) only, to amitrole (aminotriazole) only, and to both types of herbicides. After a 10-year latency was achieved, 3 stomach cancer deaths were observed vs. 0.71 expected ($p<0.05$). None were attributable to amitrol alone, but two were assigned to phenoxy acids alone while the remaining stomach cancer death occurred in a worker exposed to both amitrol and phenoxy acids. The excess was more pronounced (3 observed vs. 0.57 expected, $p<0.05$) among those with early exposure (1957-1961) to phenoxy acids and/or amitrol. If persons who were exposed to just amitrol alone are excluded, thus leaving individuals exposed to phenoxy acid alone and amitrol in combination, the excess is enhanced further (3 observed vs. 0.41 expected, $p<0.01$).

Axelson et al. (1980) also noted an excess in total "tumors" after 10 years latency as well (15 observed vs. 6.87 expected, $p<0.005$). This is pronounced in those exposed early to phenoxy acids alone (6 observed vs. 2.60 expected, $p<0.01$) and phenoxy acids in combination with amitrol (5 observed vs. 1.34 expected, $p<0.05$). Presumably, "tumors" in Sweden are analogous to malignant neoplasms in the United States. The author states that no specific type of tumor predominates and no breakdown by tumor type is provided.

The other study showing increased stomach cancer mortality is the follow-up of 75 workers exposed to 2,3,7,8-TCDD during and after a 1953 runaway reaction at a trichlorophenol manufacturing facility in Ludwigshafen, Federal Republic of Germany (Thiess and Frentzel-Beyme, 1977). Two sources were used to calculate expected deaths: national mortality rates for the period 1971-1974, and 1972-1975 rates for Rhinehessen-Palatinate, the region in which Ludwigshafen is located.*

The results, shown in Table 11-32, indicate an increased rate of stomach cancer mortality that also is not likely to have been due to chance alone.

Two aspects of the methodology used could have influenced these results. First, the available report does not include an analysis allowing for a minimum period of cancer induction. All three stomach cancer deaths in the Ludwigshafen cohort occurred more than 10 years after initial exposure. Employing a 10-year restriction to follow-up (as in the Swedish cohort study) would result in a higher relative risk estimate by reducing the number of expected deaths.

Secondly, national and regional mortality rates from the 1970s were used to generate expected deaths to compare with observed mortality over a much longer period (1953-1977). The substantial decline in stomach cancer mortality in West Germany during the late 1950s and 1960s would likely make these expected figures too large.

The researchers also used an internal control group that does not raise the second concern discussed above. This group consisted of 75 men, each matched to study group members by age and date of entry into employment, and selected at random from a list of over 10,000 persons who had been included in previous cohort studies by the same investigators. No stomach cancer deaths occurred in this control group during the follow-up period. Thus, use of the internal control groups also indicates an excess of stomach cancers in the exposed workers.

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*The report originally included expected deaths using rates for the city of Ludwigshafen, which were later shown to be inaccurate.

## TABLE 11-32

## Analysis of Stomach Cancer Mortality in a Group of West German Factory Workers Exposed to 2,3,7,8-TCDD*

| Source for Expected Deaths | Stomach Cancer Deaths |  | Relative Risk | Significance Level |
|---|---|---|---|---|
|  | Observed | Expected |  |  |
| Federal Republic of Germany 1971-1974 | 3 | 0.559 | 5.4 | 0.02 |
| Rhinehessen-Palatinate 1972-1975 | 3 | 0.495 | 6.1 | 0.01 |

*Source: Thiess and Frentzel-Beyme, 1977

In an update of this earlier study, Thiess et al. (1982) continued the follow-up of his cohort through 1979 by adding 2 additional years of follow-up and apparently reducing the size of his cohort from 75 to 74. Altogether 21 deaths (4 more than from the earlier study) occurred vs. 18 and 19 deaths in the 2 matched (1 to 1) internal comparison groups. With respect to cancer deaths, the numbers were respectively 7, 5 and 5. The first control group was manually matched from the total number of persons (5500 included in the cohort until the end of 1976) and the second, at random, by computer for some 8000 employees. In addition, 19 expected total deaths were estimated based on 1970-1975 mortality statistics of Rhinehessin-Palatinate, 18 expected deaths based on 1970-1975 mortality statistics of Ludwigshafen, and 20 expected deaths based upon 1971-1974 mortality statistics of the Federal Republic of Germany. Just as in the earlier study, the three stomach carcinomas noted earlier appear to be significantly elevated regardless of which external comparison group is used (Table 11-33).

On the other hand, one stomach cancer appeared in the randomized internal control group. None appeared in the manually matched internal control. No other elevated risks for any other cause were evident and no STSs appeared. When latency was considered only, the risk of stomach cancer remained significantly elevated after a lapse of 10 years (3 observed, 0.52 expected, $p<0.016$) and then after a lapse of 15 years (2 observed, 0.23 expected, $p<0.02$) based upon death rates of Rhinehessin-Palatinate, 1970-1975.

Again, these study conclusions are limited by the small size of the study group and the very few cancer deaths noted at any particular site. Thus, it is insensitive to the detection of a significantly elevated risk for most causes of cancer, especially STS and lymphomas. Although, stomach cancer is elevated significantly, it is based only upon three deaths and

## TABLE 11-33

### Reanalysis of Stomach Cancer Mortality in a Group of West German Factory Workers Exposed to 2,3,7,8-TCDD*

| Source for Expected Deaths | Stomach Cancer Deaths | | Relative Risk | Significance Level |
|---|---|---|---|---|
| | Observed | Expected | | |
| Federal Republic of Germany 1971-1974 | 3 | 0.7 | 4.3 | 0.034 |
| Rhinehessin-Palatinate 1970-1975 | 3 | 0.64 | 4.7 | 0.027 |
| Ludwigs-Shafen 1970-1975 | 3 | 0.61 | 4.9 | 0.024 |

*Source: Thiess et al., 1982

since one stomach cancer death has been noted in an internal control group in the updated version, it appears that this finding has been weakened somewhat. Furthermore, as was pointed out earlier, trends in stomach cancer mortality during the 1950s, 1960s and 1970s could make the comparison of stomach cancer mortality with expected deaths less valid based upon 1970-1975 rates.

In summary, the evidence that phenoxyacetic acids and/or 2,3,7,8-TCDD might increase the risk of stomach cancer consists of two studies, each of which reports a statistically significant excess that is based on only three stomach cancer deaths. Further follow-up of these and similar cohorts is warranted, but firm conclusions cannot yet be made.

Four additional cohort studies have reported results that do not show increased stomach cancer mortality rates in groups of workers exposed to phenoxyacetic acids and/or 2,3,7,8-TCDD. These are studies of 2,4,5-T production workers in Midland, Michigan (Ott et al., 1980), Finnish phenoxyacetic acid herbicide applicators (Riihimaki et al., 1978), the Nitro study in which workers were exposed to 2,3,7,8-TCDD (Zack and Suskind, 1980) and trichlorophenol manufacturing workers (Cook et al., 1980).

As previously mentioned, the Nitro study included a single death from STS and a weakly suggestive increase in lymphatic and hematopoietic system cancer mortality. The Midland study of 204 workers included only one cancer death, a tumor in the respiratory system. In the Finnish study, histologic information on tumor types was not provided; however, there were no deaths from lymphoma.

The results pertinent to stomach cancer mortality in the three studies are shown in Table 11-34. Results of neither the Midland study nor the

### TABLE 11-34

### Stomach Cancer Mortality in Three Studies of Workers Exposed to Phenoxyacetic Acid Herbicides and/or 2,3,7,8-TCDD

| Stomach Cancer Deaths | | Relative Risk | 95% Confidence Interval | Reference |
|---|---|---|---|---|
| Observed | Expected | | | |
| 0 | 0.14[a] | 0 | 0-26.3 | Ott et al., 1980 |
| 5 | 6.9[a,b] | 0.7 | 0.2-1.7 | Riihimaki et al., 1978 |
| 0 | 0.5[b] | 0 | 0-7.4 | Zack and Suskind, 1980 |

[a] Estimated from total cancer expected deaths (see footnote in text).

[b] Entire follow-up period without regard for minimum time for cancer induction (Ott et al., 1980 used a 10-year minimum induction period).

Nitro study contradict the findings of the Swedish and West German investigations previously discussed. This can be shown in two ways. First, the upper 95% confidence limits for the relative risk estimates from these two "negative" studies exceed even the highest point estimates of relative risk (6.1) from the two "positive" studies (see Table 11-31).

This indicates that the relative risk estimates from the Midland and Nitro studies, even though equal to zero, are nevertheless not significantly different from the estimates of 6.1, given the sample sizes, follow-up periods, age distribution and comparison group rates.

In addition, the smallest detectable relative risk in the Midland study ($\alpha = 0.05$, $\varphi = 0.2$ one-tailed Poisson test) was 21.4 (3 observed deaths, 0.14 expected).* Similarly, the smallest detectable relative risk in the Nitro study ($\alpha = 0.05$, $\varphi = 0.2$, one-tailed Poisson test) was 10.0 (5 observed deaths, 0.5 expected). This calculation is based on results for the entire follow-up period. If, as in the Midland study, a minimum period of cancer induction had been employed, the expected deaths would have been fewer and the smallest reasonably detectable relative risk would have been greater. This analysis of statistical power indicates that the Nitro and Midland studies had very low probabilities of detecting the ~6-fold increases in risk suggested by the Swedish and West German investigations.

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*Ott et al. (1980) did not report expected deaths from stomach cancers. The figure 0.14 was obtained by multiplying the numbers of expected deaths from all cancers (2.6, allowing a 10-year minimum induction period) by the percentage of stomach cancers among the expected deaths in the Nitro study (0.5/9.04 = 5.5%). The two studies used United States white male mortality rates and covered similar calendar years in follow-up (1949-1978 in Nitro and 1950-1976 in Midland), but a similarity in age distributions cannot be established from the published reports.

Statistically, the study of Finnish herbicide applicators is inconsistent with the results of the Swedish and West German cohort studies. The smallest reasonably detectable relative risk ($\alpha = 0.05$, $\varphi = 0.2$, one-tailed Poisson test) was only 3.1 (11 observed deaths, 3.6 expected).* The study, therefore, appears powerful enough to detect relative risks even smaller than those seen in the Swedish and West German studies. A partial explanation for this apparent inconsistency could lie in the fact that the Finnish study set the minimum period of herbicide exposure for membership in the cohort at 10 days (2 working weeks) and noted that the "total strength of exposure has, in most cases, been a few weeks only." The Swedish study of herbicide applicators set the minimum exposure at 46 days (>1 spraying season).

There are also certain inconsistencies in the data from the Finnish study that the authors note but find difficult to explain. In particular, no cancer deaths occurred during the latter part of the study period among Forestry Authority workers (1 of 4 groups included in the cohort), even though 9.0 deaths were expected. This finding strongly suggests some deficiency in follow-up or in the source records from which vital status was determined.

In summary, four cohort studies of workers exposed to phenoxyacetic acid herbicides and/or 2,3,7,8-TCDD do not report increased risks of stomach cancer. Only one of these, however, was statistically powerful enough to be inconsistent with the two studies that tentatively suggest an increase in stomach cancer risk. The available report of this study of Finnish herbicide applicators contains methodologic questions that require clarification.

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*The expected stomach cancer deaths were estimated in the same manner as for the Midland study. A proportion of 20% of all cancer deaths was applied because Finnish male mortality rates are known to be very high.

**11.2.3. Summary of Case Reports and Epidemiologic Studies.** By adding together the number of workers exposed to phenoxy acids and/or chlorophenols from all case studies, an unusually high number of STSs is shown, considering the rarity of the disease. This excess is suggestive of an association of cancer with exposure to phenoxy acids and/or chlorophenols, and consequently, with the impurities found in these herbicides, including 2,3,7,8-TCDD.

Two Swedish case-control studies report highly significant association of STS with exposure to phenoxy acid and/or chlorophenols. They do not pinpoint the risk to the dioxin contaminants, however. In fact, in one study, the risk was found to extend to phenoxy acids free of dioxin impurities. In that study, the risk increases to 17 when phenoxy acids known to contain dioxin impurities (polychlorinated dibenzodioxins and dibenzofurans) are considered. The extent of possible observer bias and recall bias introduced into these studies by using self-administered questionnaires is not of sufficient magnitude to have produced the highly significant risks found in the studies.

Later studies did not reveal a significant excess risk of STS. However, methodology problems make these latter studies limited with respect to evaluating the risk of STSs from exposure to phenoxy acids and/or chlorophenols and, consequently, 2,3,7,8-TCDD.

The Swedish case-control studies provide limited evidence for the carcinogenicity of phenoxy acids and/or chlorophenols in humans. However, with respect to the dioxin impurities contained therein, the evidence for the human carcinogenicity for 2,3,7,8-TCDD based on the epidemiologic studies is only suggestive because of the difficulty of evaluating the risk of 2,3,7,8-TCDD exposure in the presence of the confounding effects of phenoxy acids and/or chlorophenol.

There is less evidence incriminating 2,4,5-T and/or 2,3,7,8-TCDD as the cause of malignant lymphoma and stomach cancer in humans.

## 11.3. QUANTITATIVE ESTIMATION OF RISKS OF EXPOSURE TO 2,3,7,8-TCDD AND HxCDDs

11.3.1. Introduction. This quantitative section deals with the incremental unit risk from exposure to 2,3,7,8-TCDD and HxCDDs by inhalation and oral routes, and their potencies relative to other carcinogens that the CAG has evaluated. The incremental unit risk estimate for an air pollutant present in such small quantities as the dioxins is defined as the increased lifetime cancer risk occurring to an individual exposed continuously from birth throughout lifetime to an air concentration of 1 pg/m³ of the agent. The unit risk from oral exposure is similarly defined in terms of either µg/kg bw/day or in terms of ng/ℓ water. These calculations are done to estimate in quantitative terms the impact of the agent as a carcinogen. Unit risk estimates are used for two purposes: 1) to compare the carcinogenic potency of several agents with each other and 2) to give a crude indication of the population risk that might be associated with known (or anticipated) air or water exposure to these agents.

The incremental unit risks for both the inhalation and oral routes will be estimated from animal oral bioassays, since there are no animal inhalation studies, and none of the epidemiology studies provides sufficient exposure information for extrapolation purposes. The animal-to-man extrapolations for the oral route will assume equivalent absorption in both species. However, the unit risk for the ambient air concentration of 2,3,7,8-TCDD must be considered in terms of both its physical properties and its sources. It does not occur naturally but is emitted in small amounts from sources including the production of 2,4,5-T, trichlorophenol, silvex and hexachlorophene; the application of 2,3,7,8-TCDD-contaminated herbicides or wood preservatives; the burning of municipal waste, wood and PCBs; and, possibly, dust from 2,3,7,8-TCDD-contaminated soil.

Physically, 2,3,7,8-TCDD has a very low vapor pressure and is not normally airborne. At room temperature it is a crystalline solid, melting at 305°C. When 2,3,7,8-TCDD is present in air, it is likely to be attached to particulates, to which it strongly binds. It has been measured in air only in the vicinity of burning processes and in dust from contaminated soil, and has not been found in the general air environment.

### 11.3.2. Procedures for the Determination of Incremental Unit Risk from Animal Data and Description of the Low-Dose Animal Extrapolation Model.

Following is an abbreviated description of the procedures used in animal-to-man extrapolation. A more complete description is given in Anderson et al. (1983).

In the development of quantitative estimates of carcinogenic risk from lifetime animal studies it is assumed, unless evidence exists to the contrary, that if a carcinogenic response occurs at the dose levels used in the study, then responses will also occur at all lower doses with an incidence determined by the dose as indicated by the extrapolation model. While both TCDD and HxCDD cause cancer in animals at lower doses than any other known or suspect carcinogen, environmental levels are also extremely low. Thus, an extrapolation methodology must be employed.

There is no solid scientific basis for any mathematical extrapolation model that relates carcinogen exposure to cancer risks at the extremely low concentrations that must be dealt with in evaluating environmental hazards. Such low levels of risk cannot be measured directly either by animal experiments or by epidemiologic studies.

In the absence of any strongly suggestive evidence to the contrary for TCDD or HxCDD, the linear nonthreshold model has been adopted as the primary basis for risk extrapolation in the low-dose region of the dose-response relationship. The risk estimates made with this model should be regarded as conservative, representing the most plausible upper limit for the risk; i.e., the true risk is not likely to be higher than the estimate, but it could be lower.

The mathematical formulation chosen to describe the linear nonthreshold dose-response relationship at low doses is the linearized multistage model. It is called the linearized model because the procedure determines a linear function, $q_1^*$, consistent with the observed data in a statistical sense. Thus, the multistage model procedure employs enough arbitrary constants to be able to fit almost any monotonically increasing dose-response data, and then it incorporates a procedure for estimating the largest possible linear slope (in the 95% upper confidence limit sense) at low extrapolated doses that is consistent with the data at all dose levels of the experiment. The multistage model has the form

$$P(d) = 1 - \exp [-(q_0 + q_1 d + q_2 d^2 + \ldots + q_k d^k)]$$

where

$$q_i \geq 0, \; i = 0, 1, 2, \ldots, k$$

and P(d) = the lifetime risk (probability) of cancer at dose d.

Equivalently,

$$P_t(d) = 1 - \exp [(q_1 d + q_2 d^2 + \ldots + q_k d^k))]$$

where

$$P_t(d) = \frac{P(d) - P(0)}{1 - P(0)}$$

is the extra risk over background rate at dose d. The estimate $q_1^*$ is the 95% upper-limit on $q_1$ at lower doses. A more complete description of the model is given in Appendix B.

11.3.3. Selection of Data. For some chemicals, several studies in different animal species, strains and sexes, each run at several doses and different routes of exposure may be available. A choice must be made as to which of the data sets from several studies to use in the model. The procedures used in evaluating these data are consistent with the approach of making a maximum-likely risk estimate. They are listed below as follows:

1. The tumor incidence data are separated according to organ sites or tumor types. The set of data (i.e., dose and tumor incidence) used in the model is the set where the incidence is statistically significantly higher than the control for at least one test dose level and/or where the tumor incidence rate shows a statistically significant trend with respect to dose level. The data set that gives the highest estimate of the lifetime carcinogenic risk, $q_1$*, is selected in most cases. However, efforts are made to exclude data sets that appear to have produced spuriously high risk estimates because of a small number of animals. That is, if two sets of data show a similar dose-response relationship, and one has a very small sample size, the set of data having the larger sample size is selected for calculating the carcinogenic potency.

2. If there are two or more data sets of comparable size that are identical with respect to species, strain, sex and tumor sites, the geometric mean of $q_1$*, estimated from each of these data sets, is used for risk assessment.

   In some cases one or more of these studies may be negative, but the 95% upper limit $q_1$* will still be greater than zero.

3. If two or more significantly increased tumor sites are observed in the same study, and if the data are available, the number of animals with at least one of the specific tumor sites under consideration is used as incidence data in the model. Alternatively, the total number of significant tumors may also be used in some cases.

11.3.4. Calculation of Human Equivalent Dosages for Animal-to-Man Extrapolation. It is appropriate to correct for metabolism differences between species and absorption factors through different routes of administration.

Following the suggestion of Mantel and Schneiderman (1977), it is assumed that mg/surface area/day provides an equivalent dose between species. To a close approximation, since the surface area is proportional to the 2/3 power of the weight, as would be the case for a perfect sphere, the exposure in mg/day per 2/3 power of the weight is also considered to be equivalent exposure. In an animal experiment, this equivalent dose is computed in the following manner.

Let

$L_e$ = duration of experiment

$l_e$ = duration of exposure

m = average dose/day in mg during administration of the agent (i.e., during $l_e$) and

W = average weight of the experimental animal

Then, the lifetime average exposure is

$$d = \frac{l_e \times m}{L_e \times W^{2/3}}$$

A more expanded discussion is given in Anderson et al. (1983).

**11.3.5. Alternative Methodological Approaches.** The methods used by the CAG for quantitative assessment are consistently conservative, i.e., tending toward high estimates of risk. The most important part of the methodology contributing to this conservatism in this respect is the linear nonthreshold extrapolation model. There are a variety of other extrapolation models that could be used, most of which would give lower risk estimates. These alternative models have not been used by the CAG in the following analysis, but three are included for comparison in the appendix. The models presented there are the one-hit, probit and Weibull models. The CAG feels that with the limited data available from these animal bioassays, most of which are conducted at high dosage levels, almost nothing is known about the true shape of the dose response curve at low environmental levels. The position is taken by the CAG that the risk estimates obtained by use of the linear nonthreshold model are upper limits, and the true risk could be lower.

Another modification of the method described here involves the choice of the specific animal bioassay as the basis for extrapolation. The present approach is to use the most sensitive responder. Alternatively, the average responses of all of the adequately tested bioassay animals could be used, and then some confidence limits placed on this estimate.

Extrapolations from animals to humans could also be done on the basis of relative weights rather than surface areas. The latter approach, used here, has more basis in human pharmacological responses; it is not clear which of the two approaches is more appropriate for carcinogens. In the absence of information on this point, it seems appropriate to use the most generally accepted method, which also is more conservative. In the case of 2,3,7,8-TCDD and HxCDD gavage studies, the use of extrapolation based on surface area rather than weights increases the incremental unit risk estimates by a factor of 5.8 for rats and about 13 for mice.

11.3.6. Interpretation of Quantitative Estimates. The incremental unit risk estimate based on animal bioassays is an approximation to the excess risk in populations exposed to known carcinogen concentrations. This is because there may be important species differences in uptake, metabolism and organ distribution of carcinogens, as well as species differences in target site susceptibility, immunological responses, hormone function, and dietary factors and other diseases. The concept of equivalent doses for humans compared with animals on a mg/surface area basis has little experimental verification regarding carcinogenic response. Human populations are more variable than laboratory animals with respect to genetic constitution and diet, living environment, activity patterns and other cultural factors.

The unit risk estimate can give an indication of the relative response per unit dose ("potency") of a given agent compared with other carcinogens. The comparative potency of different agents should be more reliable when the comparison is based on studies in the same test species, strain and sex, and by the same route of exposure.

The quantitative aspect of the carcinogen risk assessment is included here because it may be of use in the regulatory decision-making process, for example, setting regulatory priorities and evaluating the adequacy of technology-based controls. However, the estimation of cancer risks to humans at low levels of exposure is uncertain. At best, the linear extrapolation model used here provides a rough but plausible estimate of the upper limit of risk; i.e., it is not likely that the true risk would be much more than the estimated risk, but it could very well be considerably lower. The risk estimates presented in subsequent sections should not be regarded as an accurate representation of the true cancer risks even when the exposures are accurately defined. The estimates presented may be factored into regulatory decisions to the extent that the concept of upper risk limits is found to be useful.

11.3.7. Incremental Unit Risk Estimates for 2,3,7,8-TCDD via the Oral and Inhalation Routes. The positive animal cancer data available for calculating an incremental unit risk estimate for 2,3,7,8-TCDD are presented in Appendix B in Tables B-1 through B-5. These are as follows:

1. The Dow (1978) diet study on Sprague-Dawley rats, Spartan substrain. Significantly increased cancers in the males included stratified squamous cell carcinomas of the tongue and squamous cell carcinomas of the nasal turbinates and hard palate. Both the original pathological analysis (Kociba) and that of an independent reviewer (Squire) are presented (Table B-1). Significant cancers in the females included lung, nasal turbinate and hard palate cancers, and liver tumors (Table B-2). As with the males, the total number of animals with at least one of these significant tumors was recorded.

2. The NCI gavage study in Osborne-Mendel rats and B6C3F1 mice.

   a. 2,3,7,8-TCDD in male rats caused an increase in follicular cell adenomas and carcinomas combined of the thyroid. However, these tumors were not considered biologically significant for risk assessment purposes. In females, the combined neoplastic nodules and hepatocellular carcinomas were considered significant (Table B-3), and these data were used. The adrenal cortical adenomas or carcinomas were not considered biologically significant.

   b. 2,3,7,8-TCDD in male mice caused an increase in hepatocellular carcinomas and in combined hepatocellular adenomas and carcinomas (Table B-4). In female mice, 2,3,7,8-TCDD caused an increase in subcutaneous tissue fibrosarcomas, lymphomas or leukemias of the hematopoietic system, liver hepatocellular carcinomas and adenomas, and thyroid follicular cell adenomas (Table B-5).

The above data have been fit by the linearized multistage model described in Section 11.3.2. These results are presented in Appendix B in some detail in Tables B-6 through B-12, and summarized in Table B-13. The results of all estimates are within an order of magnitude, with the upper-limit estimates lowest for the Dow male rats, higher for the NCI study, both rats and mice, and highest for the combined tumor sites of the female rats in the Dow study. The data from which the steepest slope factor ($q_1$*) (i.e., greatest potency) was calculated were from the Squire review of the slides. A summary of Squire's review is presented in Table B-2 and the results of the linearized multistage model extrapolation procedure are presented in Table B-9. An examination of Table B-9 shows that the high-dose group in the study was eliminated because its inclusion resulted in a poor fit of the model ($p<0.01$). A second analysis of the female rat data adjusted for early increased mortality in the high-dose group by eliminating all animals that died during the first year, so that the first tumors considered were those detected during the 13th month of the study. The results of the analysis from this adjustment are presented in Tables B-8A and B-9A.

The results yield acceptable fits of the data without dropping the responses at the highest dose levels, and these results were chosen for the final incremental unit risk estimates. The slope estimates for the Kociba (Table B-8A) and Squire (Table B-9A) analyses, $1.51 \times 10^5$ and $1.61 \times 10^5$ (mg/kg/day)$^{-1}$, were averaged by taking the geometric mean, and the final estimate thus becomes

$$q_1^* = [(1.51 \times 10^5) \times (1.61 \times 10^5)]^{1/2} = 1.56 \times 10^5 \text{ (mg/kg/ day)}^{-1}.$$

This estimate is about one-third that derived from the Squire review in Table B-8.

This upper-limit estimate represents a range of uncertainty that is related as much to the fitting procedure as to the model itself. The dropping of the highest dose-response data and the resulting increased 95% upper-limit slope estimate based on the Squire analysis can be defended on the basis that the highest dose data in this bioassay is 100 times that of the lowest, and would therefore contain very little information about the shape of the dose-response curve at low dose levels. It could also be argued on the basis of a saturation effect of either dose or response; the data can partially support either hypothesis. An adjustment of the multistage model needed to incorporate such an effect or effects, however, is felt to be unwarranted by the sparsity of the supporting evidence. As an alternative, to incorporate this uncertainty, a range of 95% upper-limit estimates of $q_1^* = 9.0 \times 10^4$ to $4.25 \times 10^5$ (mg/kg/day)$^{-1}$ has been chosen to accommodate this unusual data set.

In order to estimate an incremental unit risk for a 1 ng/ℓ concentration in drinking water, the following conversion is used:

$$1 \text{ µg/kg/day} \times 70 \text{ kg} \times 10^3 \text{ ng/µg} \times 1 \text{ day}/2 \text{ ℓ} = 3.5 \times 10^4 \text{ ng/ℓ}$$

based on human consumption of 2 ℓ water/day for a lifetime. Therefore, the incremental unit risk corresponding to 1 ng 2,3,7,8-TCDD/ℓ water is

$$q_1^* = 1.56\times10^{2}\ (\mu g/kg/day)^{-1} \times \frac{1\ \mu g/kg/day}{3.5\times10^{4}\ ng/\ell} = 4.5\times10^{-3}\ (ng/\ell)^{-1}$$

Similarly, the lower and upper limits of the range vary from $q_1^* = 2.6\times10^{-3}$ to $1.2\times10^{-2}\ (ng/\ell)^{-1}$.

This incremental unit risk estimate from an oral study must be transformed before an estimate can be made from exposure to 2,3,7,8-TCDD in the ambient air. Exposure will be assumed to occur only through respiration of 2,3,7,8-TCDD-contaminated particulates. The amount of exposure depends on the particulate size distribution. Based on the report of the International Commission on Radiological Protection (ICRP, 1959), it can be assumed that 100% of particulates of <0.1 micron in size pass the nasopharyngeal (upper respiratory tract) barrier and are deposited on the tracheobronchial and alveolar passages. For the larger-sized particles, the percentage deposition of 5-micron particles in the lower respiratory tract is not more than 30%. Even those larger particles retained by the upper respiratory tract, however, may be swallowed and eventually absorbed by ingestion. In the absence of specific data on the size distribution and eventual fate of the particles, the information developed by the ICRP, Committee 2, will be used. The Committee developed the following estimates for retention of particulate matter in the lungs. For compounds not readily soluble, 25% will be exhaled, 50% will be deposited in the upper respiratory passages and subsequently swallowed, and the final 25% will be deposited in the lungs (lower respiratory passages). Of this final 25%, half is eliminated from the lungs and swallowed in the first 24 hours, making a total of 62.5% swallowed; the remaining 12.5% remains in the lung alveoli for long periods of time; eventually some are transferred to pulmonary lymph nodes.

If we take a worst-case estimate and assume that all of the swallowed material is eventually absorbed into the body, then 75% of the inhaled material will be absorbed. We further assume a breathing rate of 20 $m^3$/day for a 70 kg man. Given these assumptions and the fact that one picogram is equal to $10^{-9}$ mg, the lifetime cancer risk for an ambient concentration of 1 pg/$m^3$ of 2,3,7,8-TCDD is $3.3 \times 10^{-5}$, as calculated below:

$$q_1^*(\text{resp.}) = 1.56 \times 10^5\ (\text{mg/kg/day})^{-1} \times 1 \times 10^{-9}\ \text{mg/pg} \times .75 \times 20\ \text{m}^3/70\ \text{kg}$$

or

$$q_1^*(\text{resp.}) = 3.3 \times 10^{-5}\ (\text{pg/m}^3)^{-1}.$$

Similary, the range of estimates is $1.9 \times 10^{-5}$ to $9.1 \times 10^{-5}$ $(\text{pg/m}^3)^{-1}$.

11.3.8. Incremental Unit Risk Estimate for HxCDDs (1,2,3,6,7,8 and 1,2,3,7,8,9) Via the Oral and Inhalation Routes. The results of the National Toxicology Program (NTP) gavage study on a mixture of 1,2,3,6,7,8- and 1,2,3,7,8,9-HxCDD showed positive results for male and female rats (combined liver neoplastic nodules or hepatocellular carcinomas), the greater response being in the females. In the females, carcinomas appeared only in the high-dose group. In the male rats, there was also a definite trend in neoplastic nodules and carcinomas combined, but this was only marginally significant. These results are presented in Table 11-35, which includes the recent NTP reevaluation of the female rat liver slides. The review shows responses in the range of 50% less than that of the original analysis. The responses for neoplastic nodules and combined nodules and carcinomas are still statistically significant. These results have been detailed in the qualitative section of this document.

## TABLE 11-35

## NTP HxCDD (Gavage) Bioassay (NTP, 1980d)

## Osborne-Mendel Rats (2 years)

## Incidences of Neoplastic Nodules and Hepatocellular Carcinomas

| Tumor | Vehicle Control | Untreated Control | µg/kg/week | | | Estimates[a] of $q_1^*$ (µg/kg/day)$^{-1}$ |
|---|---|---|---|---|---|---|
| | | | Low-Dose 1.25 | Mid-Dose 2.5 | High-Dose 5 | |
| MALE (700 g)[b] | | | | | | |
| Number of animals examined | 74 | 75 | 49 | 50 | 48 | -- |
| Hepatocellular carcinoma (HC) | 0 | 0 | 0 | 0 | 1(2%) | -- |
| Neoplastic nodule (NN) | 0 | 2(3%) | 0 | 1(2%) | 3(6%) | $5.6\times10^{-1}$ |
| HC + NN combined | 0 | 2(3%) | 0 | 1(2%) | 4(8%)[c] | $5.9\times10^{-1}$ |
| Human equivalent dose µg/kg/day | 0 | 0 | 0.04 | 0.08 | 0.15 | -- |

TABLE 11-35 (cont.)

| Tumor | Vehicle Control | Untreated Control | μg/kg/week Low-Dose 1.25 | μg/kg/week Mid-Dose 2.5 | μg/kg/week High-Dose 5 | Estimates[a] of $q_1^*$ (μg/kg/day)$^{-1}$ |
|---|---|---|---|---|---|---|
| | | | FEMALE (450 g)[d] | | | |
| Number of animals examined | 75 | 73 | 50 | 50 | 50 | -- |
| Hepatocellular carcinoma (HC) | 0 | 0 | 0 | 0 | 2(4%) | 3.2x10$^{-1}$ |
| Neoplastic nodule (NN) | 2(3%) | 1(1%) | 5(10%) | 7(14%)[c] | 16(32%)[e] | 3.3 |
| HC + NN combined | 2(3%) | 1(1%) | 5(10%) | 7(14%)[c] | 18(36%)[e] | 3.5 |
| Human equivalent dose μg/kg/day | 0 | 0 | 0.03 | 0.06 | 0.12 | -- |

[a] 95% upper-limit estimate of linear term in the multistage model based on human equivalent dosages using surface area correction.

[b] Analysis by NTP (1980d)

[c] $p<0.05$ versus vehicle-control

[d] Reevaluation by Hildebrandt (1983)

[e] $p<0.001$ versus vehicle-control

In female mice, there was a dose-related trend in hepatocellular carcinomas, but only the combined adenomas and carcinomas were significant. In male mice, there was a minor trend in hepatocellular adenomas, but no increase, statistical or otherwise, in hepatocellular carcinomas (Table 11-36).

Although no statistically significant increase in carcinomas occurred in mice or rats of either sex, when neoplastic nodules in the rats and hepatocellular adenomas in the mice were included in the data, the results became significant for all groups. These combined results were then fitted to the multistage model for all four groups. As shown in Tables 11-35 and 11-36, the 95% upper-limit unit risk estimates are as follows:

| | | |
|---|---|---|
| Rat - | male | $q_1^* = 0.59\ (\mu g/kg/day)^{-1}$ |
| | female | $q_1^* = 3.5\ (\mu g/kg/day)^{-1}$ |
| Mouse - | male | $q_1^* = 11.0\ (\mu g/kg/day)^{-1}$ |
| | female | $q_1^* = 2.9\ (\mu g/kg/day)^{-1}$ |

The usual CAG procedure is to use the most sensitive sex-species for estimating the 95% upper-limit unit risk. Under that procedure, which is based on the linearized multistage model with surface area correction for animal-to-man extrapolation, the male mouse data base yielding a $q_1^*$ = 11.0 $(\mu g/kg/day)^{-1}$ would be selected to provide the upper limit estimate of potency. However, as examination of Tables 11-35 and 11-36 show, there are several reasons to give weight to the female rat data base also. These are as follows: 1) low spontaneous (control) rates in the rat vs. the male mouse liver; 2) statistically significant increases in both the mid and high level dose groups vs. control for the female rat; the male mouse response was significant only at the high dose; 3) a more distinct dose response trend in the female rat vs. the male mouse; and 4) the only hepatocellular carcinomas in the female rat were in the high dose group. There were none in 148 control animals. By comparison, the male mouse showed no clear trend in carcinomas.

## TABLE 11-36

### NTP HxCDD (Gavage) Bioassay (NTP, 1980d)

### B6C3F1 Mice (104 weeks)

### Incidences of Adenomas and Hepatocellular Carcinomas

| Tumor | Vehicle Control | Untreated Control | µg/kg/week Low-Dose 1.25 | µg/kg/week Mid-Dose 2.5 | µg/kg/week High-Dose 5 | Estimates of $q_1^{*a}$ $(\mu g/kg/day)^{-1}$ |
|---|---|---|---|---|---|---|
| | | | MALES | | | |
| Number of animals examined | 73 | 75 | 50 | 49 | 48 | -- |
| Hepatocellular carcinoma (HC) | 8(11%) | 12(16%) | 9(18%) | 5(10%) | 9(19%) | 3.71 |
| Hepatocellular adenoma (HA) | 7(10%) | 15(20%) | 5(10%) | 9(18%) | 15(31%)[b] | 6.99 |
| Combined HA and HC | 15(21%) | 27(36%) | 14(29%) | 14(29%) | 24(50%)[c] | 11.00 |
| Human equivalent daily dose (µg/kg/day) | 0 | 0 | 0.014 | 0.027 | 0.054 | -- |

TABLE 11-36 (cont.)

| Tumor | Vehicle Control | Untreated Control | µg/kg/week Low-Dose 2.5 | Mid-Dose 5.0 | High-Dose 10.0 | Estimates of $q_1^{*a}$ $(\mu g/kg/day)^{-1}$ |
|---|---|---|---|---|---|---|
| | | | FEMALES | | | |
| Number of animals examined | 73 | 74 | 48 | 47 | 47 | -- |
| Hepatocellular carcinoma (HC) | 1(1%) | 0 | 0 | 2(4%) | 2(4%) | $9.5\times10^{-1}$ |
| Hepatocellular adenoma (HA) | 2(3%) | 2(3%) | 4(8%) | 4(9%) | 9(19%)[b] | 2.61 |
| Combined HA and HC | 3(4%) | 2(3%) | 4(8%) | 6(13%) | 10(23%)[b] | 2.94 |
| Human equivalent daily dose (µg/kg/day) | 0 | 0 | 0.027 | 0.054 | 0.107 | -- |

[a] 95% upper-limit estimate of linear term in the multistage model based on human equivalent dosages using surface area correction.

[b] $p<0.01$ versus vehicle-control

[c] $p<0.001$

In addition to the above reasoning, we point to the uncertainty of the surface area correction. Nearly all the quantitative increase in the estimate of the 95% upper limit risk of the male mouse vs. the female rat (11.0/3.5 = 3.1) can be attributed to the surface area correction in the extrapolation procedure, which is greater for mice than for rats by a factor of 2.5. The surface area correction is an assumption used in the HxCDD analysis but neither supported nor contradicted by data.

Finally, for 2,3,7,8-TCDD, the female rat (different strain) has been shown to be more sensitive than the mouse even with the surface area correction.

Based on the above qualifications, the CAG has decided to modify its procedure slightly and to take the geometric mean of the 95% upper-limit estimates from the male mouse and the female rat. The final estimate is

$$q_1^* = (3.5 \text{x} 11.0)^{1/2} = 6.2\ (\mu g/kg/day)^{-1}$$

In terms of exposure to 1 µg/ℓ of HxCC contaminate and 2 ℓ/day for a lifetime, we use the same assumptions as with 2,3,7,8-TCDD:

$$1\ \mu g/kg/day = 3.5 \text{x} 10^{4}\ ng/\ell.$$

Thus, for 1 ng/ℓ in the drinking water the estimate of incremental risk is

$$P = 1 - e^{-6.2/3.5 \text{x} 10^{-4}} = 1.8 \text{x} 10^{-4}$$

In terms of continuous lifetime exposure to ambient air containing 1 pg/m³ HxCDD, the transformation as was done before with 2,3,7,8-TCDD, is

$$q_1^*(\text{HxCDD})\ (\text{resp.}) = 6.2 \text{x} 10^{3}\ (mg/kg/day)^{-1} \text{ x } 1 \text{x} 10^{-9}\ mg/pg \text{ x } 0.75 \text{x} 20\ m^{3}/70\ kg$$

$$q_1^*(\text{HxCDD})\ (\text{resp.}) = 1.3 \text{ x } 10^{-6}\ (pg/m^{3})^{-1}.$$

11.3.9. Relative Potency. One of the uses of unit risk is to compare the relative potencies of carcinogens. Potency is defined for this purpose as the linear portion of the dose-response curve, which was used to calculate the unit risk factors. To estimate the relative potency on a per-mole basis, the unit risk slope factor is multiplied by the molecular weight, and the resulting number is expressed in terms of $(\text{mMol/kg/day})^{-1}$. This is called the "relative potency index."

Figure 11-2 is a histogram representing the frequency distribution of potency indices of 55 chemicals evaluated by the CAG as suspect carcinogens. The actual data summarized by the histogram are presented in Table 11-37. Where human data are available for a compound, they have been used to calculate the index. When no human data are available, animal oral studies have been used in preference to animal inhalation studies, since animal oral studies have been conducted on the majority of these chemicals; this allows potency comparisons by route.

The potency index for 2,3,7,8-TCDD based on liver, lung and nasal turbinate and hard palate tumors in the female rat in the Dow 2,3,7,8-TCDD feeding study (Kociba et al. (1978a) is $5\times10^{7}$ $(\text{mMol/kg/day})^{-1}$. This number is derived by multiplying as follows: the 95% upper-limit slope estimate from the Dow study using the geometric mean of the Squire and Kociba analyses, $q_1^* = 1.56\times10^{5}$ $(\text{mg/kg/day})^{-1}$, by the molecular weight of 322. Rounding off to the nearest order of magnitude gives a log 10 value of 8, which is the scale presented on the horizontal axis of Figure 11-2. The index of $5\times10^{7}$ is the most potent of 55 chemicals that the CAG has evaluated as suspect carcinogens. It is 50 times more potent than the third most potent chemical, bis(chloromethyl) ether, and 50,000,000 times as potent as vinyl chloride. The potency index of HxCDD, based on combined hepatocellular adenomas and carcinomas in male mice in the NTP gavage study (NTP, 1980d), and combined nodules and hepatocellular carcinomas in female rats by gavage (NTP, 1980d) is $2.4\times10^{+6}$ $(\text{mMol/kg/day})^{-1}$. This is derived by

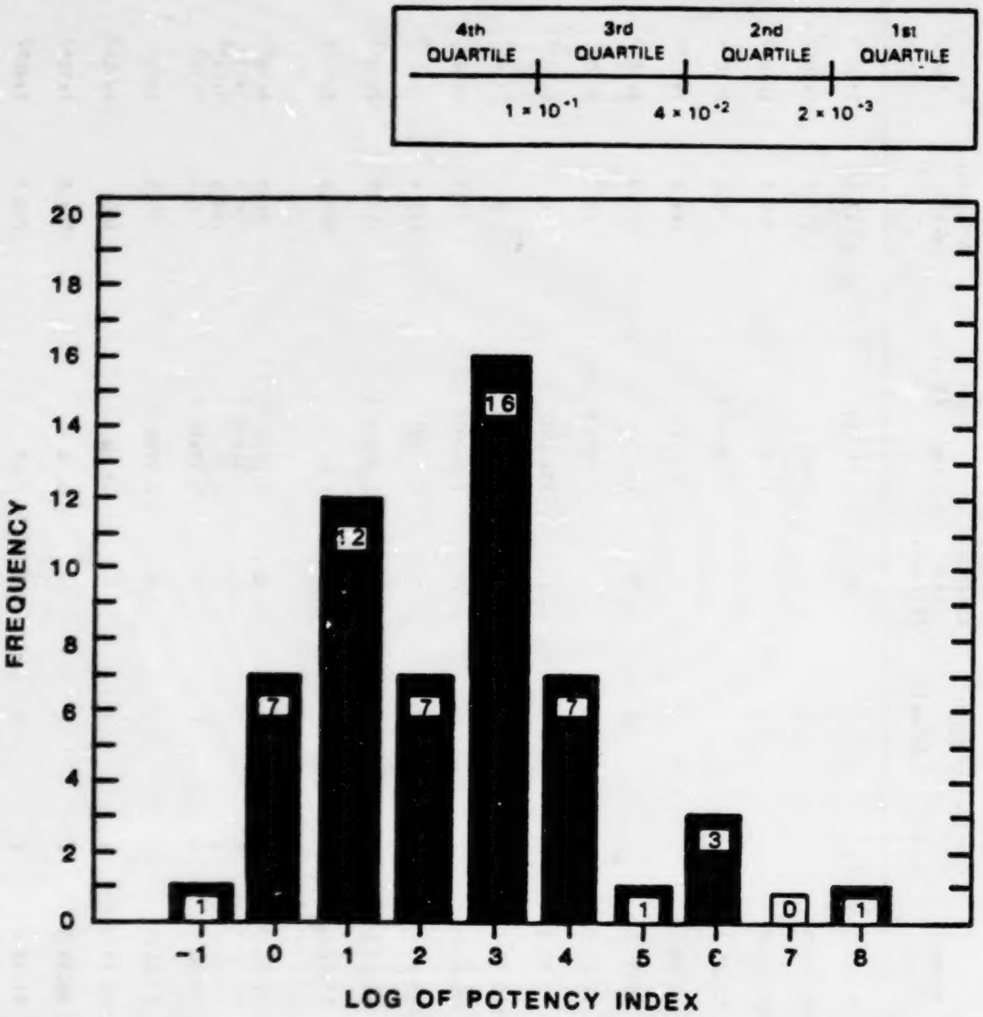

FIGURE 11-2

Histogram Representing the Frequency Distribution of the Potency Indices of 55 Suspect Carcinogens Evaluated by the Carcinogen Assessment Group

## TABLE 11-37

### Relative Carcinogenic Potencies Among 55 Chemicals Evaluated by the Carcinogen Assessment Group as Suspect Human Carcinogens

| Compounds | CAS Number | Level of Evidence[a] |  | Grouping Based on IARC Criteria | Slope[b] $(mg/kg/day)^{-1}$ | Molecular Weight | Potency Index[c] | Order of Magnitude ($\log_{10}$ index) |
|---|---|---|---|---|---|---|---|---|
|  |  | Humans | Animals |  |  |  |  |  |
| Acrylonitrile | 107-13-1 | L | S | 2A | 0.24 (W) | 53.1 | $1 \times 10^{+1}$ | +1 |
| Aflatoxin $B_1$ | 1162-65-8 | L | S | 2A | 2900 | 312.3 | $9 \times 10^{+5}$ | +6 |
| Aldrin | 309-00-2 | I | L | 3 | 11.4 | 369.4 | $4 \times 10^{+3}$ | +4 |
| Allyl chloride | 107-05-1 |  |  |  | $1.19 \times 10^{-2}$ | 76.5 | $9 \times 10^{-1}$ | 0 |
| Arsenic | 7440-38-2 | S | I | 1 | 15 (H) | 149.8 | $2 \times 10^{+3}$ | +3 |
| B[a]P | 50-32-8 | I | S | 2B | 11.5 | 252.3 | $3 \times 10^{+3}$ | +3 |
| Benzene | 71-43-2 | S | S | 1 | $2.9 \times 10^{-2}$ (W) | 78 | $2 \times 10^{0}$ | 0 |
| Benzidene | 92-87-5 | S | S | 1 | 234 (W) | 184.2 | $4 \times 10^{+4}$ | +5 |
| Beryllium | 7440-41-7 | L | S | 2A | 2.6 (W) | 9 | $2 \times 10^{+1}$ | +1 |
| 1,3-Butadiene | 106-99-0 | I | S | 2B | $1.0 \times 10^{-1}$ (I) | 54.1 | $5 \times 10^{0}$ | +1 |
| Cadmium | 7440-43-9 | L | S | 2A | 6.1 (W) | 112.4 | $7 \times 10^{+2}$ | +3 |
| Carbon tetrachloride | 56-23-5 | I | S | 2B | $1.30 \times 10^{-1}$ | 153.8 | $2 \times 10^{+1}$ | +1 |
| Chlordane | 57-74-9 | I | L | 3 | 1.61 | 409.8 | $7 \times 10^{+2}$ | +3 |
| Chlorinated ethanes |  |  |  |  |  |  |  |  |
| 1,2-Dichloroethane | 107-06-2 | I | S | 2B | $9.2 \times 10^{-2}$ | 98.9 | $9 \times 10^{0}$ | +1 |
| Hexachloroethane | 67-72-1 | I | L | 3 | $1.42 \times 10^{-2}$ | 236.7 | $3 \times 10^{0}$ | 0 |
| 1,1,2,2-Tetrachloroethane | 79-34-5 | I | L | 3 | 0.20 | 167.9 | $3 \times 10^{+1}$ | +1 |
| 1,1,2-Trichloroethane | 79-00-5 | I | L | 3 | $5.73 \times 10^{-2}$ | 133.4 | $8 \times 10^{0}$ | +1 |
| Chloroform | 67-66-3 | I | S | 2B | $8.1 \times 10^{-2}$ | 119.4 | $1 \times 10^{1}$ | +1 |
| Chromium VI | 7440-47-3 | S | S | 1 | 41 (W) | 100 | $4 \times 10^{+3}$ | +4 |
| DDT | 50-29-3 | I | S | 2B | 0.34 | 354.5 | $1 \times 10^{+2}$ | +2 |
| Dichlorobenzidine | 91-94-1 | I | S | 2B | 1.69 | 253.1 | $4 \times 10^{+2}$ | +3 |

TABLE 11-37 (cont.)

| Compounds | CAS Number | Level of Evidence[a] Humans | Level of Evidence[a] Animals | Grouping Based on IARC Criteria | Slope[b] $(mg/kg/day)^{-1}$ | Molecular Weight | Potency Index[c] | Order of Magnitude ($\log_{10}$ index) |
|---|---|---|---|---|---|---|---|---|
| 1,1-Dichloroethylene (Vinylidene chloride) | 75-35-4 | I | L | 3 | 1.16 (I) | 97 | $1\times10^{+2}$ | +2 |
| Dichloromethane (Methylene chloride) | 75-09-2 | I | S | 2B | $1.4\times10^{-2}$ (I) | 84.9 | $1\times10^{0}$ | 0 |
| Dieldrin | 60-57-1 | I | S | 2B | 30.4 | 380.9 | $1\times10^{+4}$ | +4 |
| 2,4-Dinitrotoluene | 121-14-2 | I | S | 2B | 0.31 | 182 | $6\times10^{+1}$ | +2 |
| Diphenylhydrazine | 122-66-7 | I | S | 2B | 0.77 | 180 | $1\times10^{+2}$ | +2 |
| Epichlorohydrin | 106-89-8 | I | S | 2B | $9.9\times10^{-3}$ | 92.5 | $9\times10^{-1}$ | 0 |
| Bis(2-chloroethyl)ether | 111-44-4 | I | S | 2B | 1.14 | 143 | $2\times10^{+2}$ | +2 |
| Bis(chloromethyl)ether | 542-88-1 | S | S | 1 | 9300 (I) | 115 | $1\times10^{+6}$ | +6 |
| Ethylene dibromide (EDB) | 106-93-4 | I | S | 2B | 41 | 187.9 | $8\times10^{+3}$ | +4 |
| Ethylene oxide | 75-21-8 | L | S | 2A | $3.5\times10^{-1}$ (I) | 44.1 | $2\times10^{+1}$ | +1 |
| Heptachlor | 76-44-8 | I | S | 2B | 3.37 | 373.3 | $1\times10^{+3}$ | +3 |
| Hexachlorobenzene | 118-74-1 | I | S | 2B | 1.67 | 284.4 | $5\times10^{+2}$ | +3 |
| Hexachlorobutadidne | 87-68-3 | I | L | 3 | $7.75\times10^{-2}$ | 261 | $2\times10^{+1}$ | +1 |
| Hexachlorocyclohexane |  |  |  |  |  |  |  |  |
| technical grade |  |  |  |  | 4.75 | 290.9 | $1\times10^{+3}$ | +3 |
| alpha isomer | 319-84-6 | I | S | 2B | 11.12 | 290.9 | $3\times10^{+3}$ | +3 |
| beta isomer | 319-85-7 | I | L | 3 | 1.84 | 290.9 | $5\times10^{+2}$ | +3 |
| gamma isomer | 58-89-9 | I | L | 3 | 1.33 | 290.9 | $4\times10^{+2}$ | +3 |
| Hexachlorodibenzodioxin 1,2,3,6,7,8- and 1,2,3,7,8,9- | 34465-46-8 | I | S | 2B | $6.2\times10^{+3}$ | 391 | $2\times10^{+6}$ | +6 |
| Nickel refinery dust |  | S | S | 1 | 1.05 (W) | 240.2 | $2.5\times10^{+2}$ | +2 |
| Nickel subsulfide | 0120-35-722 | S | S | 1 | 2.1 (W) | 240.2 | $5.0\times10^{+2}$ | +3 |

TABLE 11-37 (cont.)

| Compounds | CAS Number | Level of Evidence[a] |  | Grouping Based on IARC Criteria | Slope[b] $(mg/kg/day)^{-1}$ | Molecular Weight | Potency Index[c] | Order of Magnitude ($\log_{10}$ Index) |
|---|---|---|---|---|---|---|---|---|
|  |  | Humans | Animals |  |  |  |  |  |
| Nitrosamines |  |  |  |  |  |  |  |  |
| Dimethylnitrosamine | 62-75-9 | I | S | 2B | 25.9 (not by $q_1^*$) | 74.1 | $2x10^{+3}$ | +3 |
| Diethylnitrosamine | 55-18-5 | I | S | 2B | 43.5 (not by $q_1^*$) | 102.1 | $4x10^{+3}$ | +4 |
| Dibutylnitrosamine | 924-16-3 | I | S | 2B | 5.43 | 158.2 | $9x10^{+2}$ | +3 |
| N-nitrosopyrrolidine | 930-55-2 | I | S | 2B | 2.13 | 100.2 | $2x10^{+2}$ | +2 |
| N-nitroso-N-ethylurea | 759-73-9 | I | S | 2B | 32.9 | 117.1 | $4x10^{+3}$ | +4 |
| N-nitroso-N-methylurea | 684-93-5 | I | S | 2B | 302.6 | 103.1 | $3x10^{+4}$ | +4 |
| N-nitroso-diphenylamine | 86-30-6 | I | S | 2B | $4.92x10^{-3}$ | 198 | $1x10^{0}$ | 0 |
| PCBs | 1336-36-3 | I | S | 2B | 4.34 | 324 | $1x10^{+3}$ | +3 |
| Phenols |  |  |  |  |  |  |  |  |
| 2,4,6-Trichlorophenol | 88-06-2 | I | S | 2B | $1.99x10^{-2}$ | 197.4 | $4x10^{0}$ | +1 |
| 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) | 1746-01-6 | I | S | 2B | $1.56x10^{+5}$ | 322 | $5x10^{+7}$ | +8 |
| Tetrachloroethylene | 127-18-4 | I | L | 3 | $5.1x10^{-2}$ | 165.8 | $8x10^{0}$ | +1 |
| Toxaphene | 8001-35-2 | I | S | 2B | 1.13 | 414 | $5x10^{+2}$ | +3 |
| Trichloroethylene | 79-01-6 | I | L/S | 3/2B | $1.1x10^{-2}$ | 131.4 | $1x10^{0}$ | 0 |
| Vinyl chloride | 75-01-4 | S | S | 1 | $1.75x10^{-2}$ (I) | 62.5 | $1x10^{0}$ | 0 |

[a] S = Sufficient evidence; L = Limited evidence; I = Inadequate evidence

[b] Animal slopes are 95% upper-bound slopes based on the linearized multistage model. They are calculated based on animal oral studies, except for those indicated by I (animal inhalation), W (human occupational exposure) and H (human drinking water exposure). Human slopes are point estimates based on the linear nonthreshold model. Not all of the carcinogenic potencies presented in this table represent the same degree of certainty. All are subject to change as new evidence becomes available. The slope value is an upper bound in the sense that the true value (which is unknown) is not likely to exceed the upper bound and may be much lower, with a lower bound approaching zero. Thus, the use of the slope estimate in risk evaluations requires an appreciation for the implication of the upper bound concept as well as the "weight of evidence" for the likelihood that the substance is a human carcinogen.

[c] The potency index is a rounded-off slope in $(mmol/kg/day)^{-1}$ and is calculated by multiplying the slopes in $(mg/kg/day)^{-1}$ by the molecular weight of the compound.

multiplying the mean 95% upper-limit slope factor $q_1^* = 6.2 \times 10^3$ (mg/kg/day)$^{-1}$ by the molecular weight, 391. This potency is about one-twentieth that of 2,3,7,8-TCDD, making it the second most potent of 55 chemicals that the CAG has evaluated as suspect carcinogens.

The ranking of relative potency indices is subject to the uncertainties involved in comparing a number of potency estimates for different chemicals based on varying routes of exposure in different species, using data from studies whose quality varies widely. Furthermore, all the indices are based on estimates of low-dose risk using linear extrapolation from the observational range. These indices are, therefore, not valid for the comparison of potencies in the experimental or observation range if linearity does not exist there. Nevertheless, the potency rankings of one and two for these dioxins cannot be easily dismissed.

## 11.4. SUMMARY AND CONCLUSIONS

### 11.4.1. Summary.

11.4.1.1. QUALITATIVE ASSESSMENT OF 2,3,7,8-TCDD --There are several chronic animal cancer bioassay studies of 2,3,7,8-TCDD: 1) a Dow Chemical Company (Kociba et al., 1977, 1978a) study in male and female Sprague-Dawley (Spartan substrain) rats; 2) the Van Miller et al. (1977a,b) study in male Sprague-Dawley rats; 3) the Toth et al. (1979) study in Swiss mice; 4) the National Toxicology Program (1980a,b) studies in rats and mice; 5) the Pitot et al. (1980) promotion study in rats; and 6) the Kouri et al. (1978) cocarcinogenicity study in mice.

The 1978 study by the Dow Chemical Company of male and female Sprague-Dawley rats fed 2,3,7,8-TCDD in doses of 22, 210 and 2200 ppt showed a highly statistically significant excess of hepatocellular carcinomas in female rats at the highest dose level and hepatocellular carcinomas and hepatocellular hyperplastic nodules in female rats at both the middle and high dose levels, as compared with the controls. In addition, at the high dose there were significant increases in carcinomas of the hard palate/nasal turbinates in both males and females, of the tongue in males, and of the lungs in females. The Van Miller et al. (1977a,b) study also showed some evidence of a carcinogenic response in the liver and lungs of male Sprague-Dawley rats at dosages of 1000 and 5000 ppt in the diet, even though the study used a relatively small number of animals. The Toth et al. (1979) study provides suggestive evidence that 2,3,7,8-TCDD induced an increased incidence of liver tumors in male mice (females were not tested) receiving 0.7 μg/kg/week by gavage.

In the National Cancer Institute rat study (NTP, 1980a), male and female Osborne-Mendel rats were administered 2,3,7,8-TCDD by gavage at three dose levels: 0.01, 0.05 and 0.5 μg/kg/week. 2,3,7,8-TCDD induced statistically significant increases of hepatocellular carcinomas, subcutaneous fibrosarcomas and adrenal cortical adenomas in high-dose female rats. 2,3,7,8-TCDD also induced significant increases of thyroid tumors in male rats at all dose levels.

In a companion mouse study by the National Cancer Institute (NTP, 1980a), male and female B6C3F1 mice were given 2,3,7,8-TCDD by gavage at dose levels of 0.01, 0.05 and 0.5 μg/kg/week for males and 0.04, 0.2 and 2.0 μg/kg/week for females. 2,3,7,8-TCDD induced statistically significant increases of hepatocellular carcinomas in the high-dose males and females, and thyroid tumors, subcutaneous fibrosarcomas and histiocytic lymphomas in females.

In the study by Pitot et al. (1980), 2,3,7,8-TCDD has been shown to be a potent liver cancer promoter after initiation with diethylnitrosamine.

Several tests of 2,3,7,8-TCDD as a promoter on mouse skin were negative, but Poland et al. (1982) showed that 2,3,7,8-TCDD can promote in one mouse strain. In the study by Kouri et al. (1978), 2,3,7,8-TCDD has been shown to be a potent cocarcinogen with 3-methyl chloranthrene.

2,3,7,8-TCDD is a potent inducer of arylhydrocarbon hydroxylase (AHH) in mammals. The AHH contains enzyme epoxidase that is known to mediate the formation of epoxides, that are potentially active carcinogenic metabolites. 2,3,7,8-TCDD may be metabolized in mammalian species by the reactive epoxide intermediate to dihydrodiol and further conjugated. 2,3,7,8-TCDD was found in liver and fat at the end of the 2-year rat feeding study. Significant covalent binding of 2,3,7,8-TCDD ($^{14}$C or tritium) derived radioactivity with protein has been demonstrated. Covalent binding of 2,3,7,8-TCDD ($^{14}$C or tritium) derived radioactivity with DNA is not significant in liver cells.

Currently available studies on the mutagenicity of 2,3,7,8-TCDD are inconclusive. Two bacterial systems, *Escherichia coli* and *S. typhimurium* (without metabolic activation), exhibited positive mutagenic activity. However, in another study of *S. typhimurium* (with and without metabolic activation), the results were negative.

Several epidemiological studies have been conducted that are relevant to the carcinogenicity assessment of 2,3,7,8-TCDD. Two Swedish epidemiologic case-control studies (Hardell and Sandstrom, 1979; Eriksson et al., 1979, 1981) reported a significant association between STSs and occupational exposure to phenoxyacetic acid herbicides and/or chlorophenols that contain 2,3,7,8-TCDD as an impurity. These studies indicated ~5-fold to 7-fold increases in the risk of developing soft-tissue sarcomas among people exposed only to phenoxyacetic acids and/or chlorophenols in comparison with people not exposed to these chemicals. The associations are high enough to make it unlikely that they have resulted entirely from random variation bias or confounding, although the possibility exists that recall bias may account for a small part of the excess; but not enough to account for the excessively high risks. When an attempt was made to separate exposures into two categories based on expected presence or absence of polychlorinated dibenzo-p-dioxin impurities, the relative risks were 17 and 4.2, respectively. This indicates that agents themselves, without the dioxin impurities, may be contributing to the risk of STSs as well. The nonpositive studies that seemingly do not support the finding of an elevated risk of cancer, specifically STS, suffer from a variety of methodological problems that will make such a risk impossible to detect in some and difficult to detect in others. Several of these require many more years of follow-up before a significant elevated risk of the relatively rare STS is found. Within this group of nonpositive studies are several where evidence of exposure to 2,3,7,8-TCDD is questionable at best and as such no elevated risk of STS will ever be found. On the other hand, several small-scale cohort studies with proven evidence of exposure to chemicals containing 2,3,7,8-TCDD have produced a small number of the relatively rare STS that certainly would not have been expected at the time. However, several epidemiologic studies are now in progress, the results of which are not yet available, that will provide additional epidemiologic evidence that may influence our conclusions at a later time. Another Swedish case-control study (Hardell et al., 1980, 1981) provides suggestive evidence of an increased risk of developing lymphomas resulting from occupational exposure to phenoxyacetic acids.

Two cohort studies, one by Axelson et al. (1980) and the other by Thiess and Frentzel-Beyme (1978) provide suggestive evidence that phenoxyacetic acids and/or 2,3,7,8-TCDD increase the risk of stomach cancer in humans.

Four other cohort studies by Ott et al. (1980), Riihimaki et al. (1978), Cook et al. (1980) and Zack and Suskind (1980) indicated no significantly increased risk of stomach cancer in people exposed to phenoxyacetic acids and/or chlorophenols, but two of these studies were of relatively low statistical power, and another study has certain inconsistencies requiring clarification.

11.4.1.2. QUALITATIVE ASSESSMENT OF HxCDD -- Hexachlorodibenzo-p-dioxin has been tested for carcinogenicity in rats and mice by gavage (NTP, 1980d) and by dermal application to mice (NTP, 1980b,c). In these studies, a 1:2 mixture of 1,2,3,6,7,8- and 1,2,3,7,8,9-HxCDD was tested. In the oral study, animals received HxCDD at doses of 0.0, 1.25, 2.5 or 5.0 μg/kg/week, except for female mice, which received 0.0, 2.5, 5.0 and 10.0 μg/kg/week. In both species and both sexes, only tumors of the liver occurred at a significantly greater incidence than in controls. In male rats and male and female mice, the liver tumor incidence was significantly increased over control values only in the high-dose groups, while in female rats the incidence was significantly greater at both the medium- and high-dose levels. At the request of EPA this study was audited during May-August 1985 by several scientists as to the pathologic evaluation and conduct of the study. The scientists have reconfirmed the NTP conclusions that the study provides carcinogenic evidence in both rats and mice. In the study of HxCDD carcinogenicity in mouse skin conducted by NTP (1980c), there were no treatment-related tumors in either the carcinogenicity bioassay or the tumor promotion assay using DMBA as an initiator. There are no available epidemiologic carcinogenicity studies in the published literature for HxCDD as the sole compound of concern. The mutagenic potential for HxCDD is unknown since no tests are reported in the available literature.

11.4.1.3. QUANTITATIVE ASSESSMENT OF 2,3,7,8-TCDD AND HxCDD -- Quantitative estimates of the potential carcinogenic impact on humans, due to both oral and inhalation exposure to both 2,3,7,8-TCDD and HxCDD, have been calculated. These estimates are all based on animal-to-human extrapolation procedures. The animal gavage and feeding studies provide the only data base for estimating the carcinogenic potency (unit risk) for 2,3,7,8-TCDD and HxCDD. While the epidemiology studies provide positive, although limited, evidence for carcinogenicity, the population exposures are unknown and the findings cannot be attributed to exposure to 2,3,7,8-TCDD alone. Thus the ingestion unit risks as well as the estimates for inhalation unit risk are derived from the gavage and feeding studies.

There is insufficient metabolism and pharmacokinetic information to alter the typically used assumptions regarding dose extrapolation. The reported intragastric absorption for 2,3,7,8-TCDD in rats varies from 52-86%; there are no absorption data for HxCDD. The assumptions used in both the TCDD and HxCDD unit risk estimates assume that human absorption by oral exposure is equal to that of the rat. Information regarding absorption by inhalation is totally lacking and is assumed to be 75% based on an ICRP (1959) lung uptake model. The upper limit unit risks were calculated using a multistage extrapolation model that is linear at low doses as programmed in GLOBAL 79.

For cancer risk due to oral exposures, the upper-limit quantitative incremental unit risk estimate is $q_1^* = 1.56\times10^{-1}$ $(ng/kg/day)^{-1}$, derived from the Kociba et al. (1977, 1978a) 2,3,7,8-TCDD feeding study in female rats that induced a statistically significant increased incidence of tumors in the liver, lungs, hard palate and nasal turbinates. Based on continuous lifetime exposure to 1 ng/ℓ 2,3,7,8-TCDD in drinking water, the

95% upper limit estimate of individual incremental cancer risk is $4.5\times10^{-3}$ with a range of upper limit values of $2.6\times10^{-3}$ to $1.2\times10^{-2}$, depending upon pathological interpretation and mortality correction. Based on continuous lifetime exposure to 1 pg/$m^3$ 2,3,7,8-TCDD in ambient air, the 95% upper-limit estimate of individual incremental cancer risk is $3.3\times10^{-5}$, with a range of upper-limit estimates of $1.9\times10^{-5}$ to $9.1\times10^{-5}$ depending upon pathologic interpretation and mortality correction. Since the inhalation unit risk values are based upon the observed incidence in the feeding study, an implicit assumption is made that 2,3,7,8-TCDD is as potent by inhalation as by ingestion exposure.

An upper-limit incremental unit risk estimate for a mixture of HxCDDs has been calculated from the NCI gavage study (NTP, 1980d). Based on combined liver heptacellular carcinomas and neoplastic nodules in female rats, and hepatocellular adenomas and carcinomas in male mice, $q_1^* = 6.2\times10^{-3}$ $(ng/kg/day)^{-1}$. A continuous lifetime exposure to 1 ng/ℓ of HxCDD in drinking water is estimated to result in an upper limit incremental unit risk of $1.8\times10^{-4}$. Similarly, for ambient air, a continuous lifetime exposure to 1 pg/$m^3$ of HxCDD is estimated to yield an upper-limit unit risk of $1.3\times10^{-6}$.

The cancer potency of 2,3,7,8-TCDD as represented by a potency index is also estimated relative to 54 other chemicals which the CAG has evaluated as carcinogens. The relative potency index is $5\times10^{7}$ $(mMol/kg/day)^{-1}$, making 2,3,7,8-TCDD the most potent animal carcinogen evaluated by the CAG. It is about 50 times more potent than the third most potent chemical, bis-(chloromethyl)ether and ~50,000,000 times more potent than vinyl chloride. The relative potency index for HxCDD is $2\times10^{6}$ $(mMol/kg/day)^{-1}$, making it the second most potent carcinogen, about one-twentieth the low dose potency of 2,3,7,8-TCDD.

11.4.2. Conclusions. There is evidence from chronic animal cancer bioassay studies that 2,3,7,8-TCDD and HxCDD are probable human carcinogens. There are no chronic animal cancer bioassay studies available that evaluate the carcinogenic potential for other polychlorinated dibenzo-p-dioxin compounds. The available data for 2,3,7,8-TCDD and HxCDD come from gavage and feeding studies, there being no studies available for inhalation exposure. The epidemiologic evidence for the carcinogenicity of 2,3,7,8-TCDD alone is inadequate, and there have been no epidemiologic studies, as yet, for HxCDD as the sole compound of concern.

2,3,7,8-TCDD has induced hepatocellular carcinomas in two strains of female rats and both sexes of one mouse strain, along with the induction of thyroid tumors, subcutaneous fibrosarcomas and tumors of the lung, nasal turbinates/hard palate in male rats, and tongue tumors in female rats. These effects notably occur at extremely low doses. There is evidence that 2,3,7,8-TCDD is also a promoter and a cocarcinogen. The evidence of carcinogenicity for 2,3,7,8-TCDD in animals is regarded as "sufficient" using the EPA interim weight-of-evidence classification system for carcinogens (U.S. EPA, 1984).

The human evidence for the carcinogenicity of 2,3,7,8-TCDD alone is regarded as "inadequate" using the EPA classification criteria, because of the difficulty of attributing the observed effects solely to the presence of 2,3,7,8-TCDD that occurs as an impurity in the phenoxyacetic acids and chlorophenols. However, the human evidence for the carcinogenicity of chlorinated phenoxy acetic herbicides and/or chlorophenols with chlorinated dibenzodioxin impurities is judged to be "limited" according to the EPA criteria.

The overall evidence for carcinogenicity, considering both animal and human studies, would place 2,3,7,8-TCDD alone in the B2 category of EPA's classification scheme, and 2,3,7,8-TCDD in association with the phenoxy herbicides and/or chlorophenols in the B1 category. Chemicals in category B are regarded as being "probably" carcinogenic in humans.

The EPA has, in the past, used an IARC weight-of-evidence classification scheme for evaluating carcinogenicity data. Using IARC classification criteria, the positive evidence in the rat and mouse studies, together with inadequate evidence in humans for 2,3,7,8-TCDD alone, is equivalent to an IARC 2B category, meaning that 2,3,7,8-TCDD is "probably" carcinogenic in humans. However, the overall weight-of-evidence for 2,3,7,8-TCDD in combination with chlorinated phenoxyacetic acid herbicides and/or chlorophenols would be classified as IARC 2A, meaning that chlorophenoxyacetic acid and/or chlorophenols containing 2,3,7,8-TCDD are "probably" carcinogenic in humans.

Hepatocellular tumors have been induced in mice and rats of both sexes following administration of a 1:2 mixture of 1,2,3,6,7,8- and 1,2,3,7,8,9-HxCDD. This level of carcinogenic evidence in animals would be regarded as "sufficient" according to the EPA classification scheme. Based on animal evidence and the lack of epidemiologic data, HxCDD would be placed in EPA's B2 category, which characterizes HxCDD as "probably" carcinogenic in humans. Using the IARC classification scheme, based on animal evidence and no epidemiology data, HxCDD would be considered to be in a 2B category meaning that HxCDD is "probably" carcinogenic in humans.

Assuming that 2,3,7,8-TCDD and HxCDD are carcinogenic in humans, upper bound incremental unit cancer risks have been estimated for both ingestion and inhalation exposure. The development of these unit risk estimates is for the purpose of evaluating the magnitude of the possible health impact from exposure to these compounds. The upper bound nature of these risk estimates is such that the true risk is not likely to be exceeded and may be lower.

Using the data from a feeding study with female rats, the cancer potency (unit risk per mg/kg/day) for 2,3,7,8-TCDD is $1.56 \times 10^{-1}$ $(ng/kg/day)^{-1}$. The upper limit estimate of incremental cancer risk is $4.5 \times 10^{-3}$ for a continuous lifetime exposure to 1 ng/ℓ of 2,3,7,8-TCDD in drinking water. The upper limit estimate of incremental cancer risk is $3.3 \times 10^{-5}$ for a continuous lifetime exposure to 1 $pg/m^3$ of 2,3,7,8-TCDD in ambient air.

Using data from a gavage study with female rats and male mice the cancer potency for HxCDD is $6.2 \times 10^{-3}$ $(ng/kg/day)^{-1}$. The upper limit estimate of incremental cancer risk is $1.8 \times 10^{-4}$ for a lifetime exposure to 1 ng/ℓ of HxCDD in drinking water. For ambient air a lifetime exposure to 1 $pg/m^3$ of HxCDD is estimated to have an upper limit risk of $1.3 \times 10^{-6}$.

In terms of low dose response, 2,3,7,8-TCDD and the 1:2 mixture of 1,2,3,6,7,8- and 1,2,3,7,8,9-HxCDD rank as the most potent and second most potent, respectively, carcinogens evaluated by EPA's CAG.

# 12. SYNERGISM AND ANTAGONISM

The interactions of 2,3,7,8-TCDD with other toxic substances are predominately mediated through its potent enzyme induction. 2,3,7,8-TCDD pretreatment significantly alters the metabolism of many other compounds, resulting in either potentiation or inhibition of their biological effects.

## 12.1. CHEMICAL CARCINOGENS

Synergistic and antagonistic activities of 2,3,7,8-TCDD with chemical carcinogens have been discussed in depth in Chapter 11 of this document.

## 12.2. NONCARCINOGENIC CHEMICALS

2,3,7,8-TCDD pretreatment has been observed to modify the effects of anesthetics (Greig, 1972). Adult male Porten rats were given a single oral dose of 200 μg 2,3,7,8-TCDD/kg bw 1-3 days preceding treatment with 100 mg/kg zoxazolamine hydrochloride or 150 mg/kg hexabarbitone sodium. 2,3,7,8-TCDD pretreatment resulted in a 54% decrease in the duration of the paralysis induced by zoxazolamine and a 2-fold increase in the sleeping time produced by hexabarbitone. A recent report compares the immunotoxicity of 2,3,7,8-TCDD, 2,3,7,8-TCDF and 2,3,7,8-TCDF plus 2,3,7,8-TCDD (coadministered) (Rizzardini et al., 1983). Seven days after administration of 1.2 μg/kg of 2,3,7,8-TCDD to C57B1/6J mice, sheep red blood cells were injected intraperitoneally and plaque-forming cells (PFC) in the spleen were counted 5 days later. 2,3,7,8-TCDD inhibited antibody production by 80%. In a parallel study, a dose of 2,3,7,8-TCDF was administered (10 μg/kg) and no significant immunotoxic effects were observed. Coadministration of 2,3,7,8-TCDD (1.2 μg/kg) plus 2,3,7,8-TCDF (10 μg/kg) resulted in 50% reduction in antibody production and demonstrates a significant antagonistic effect by 2,3,7,8-TCDF. Coadministration of these two isostereomers resulted in antagonistic effects with respect to the induction of hepatic microsomal cytochrome P-450 and 7-ethoxycoumarin O-deethylase. Sweeney et al. (1979) found that iron deficiency protected mice against the development of hepatocellular damage (including porphyria) normally caused by 2,3,7,8-TCDD exposure.

## 12.3. SUMMARY

Exposure to 2,3,7,8-TCDD has been observed to alter the biological response of many species to some compounds. This altered response is presumed to be the result of altered enzyme activities in tissue in which 2,3,7,8-TCDD exerts an inductive effect (vide ante, see Section 8.1.1.3.), although other mechanisms are possible (see Section 8.3.).

2,3,7,8-TCDD pretreatment increases the conversion of some chemical carcinogens to mutagens by hepatic S-9 preparations in in vitro test systems; however, exposure to 2,3,7,8-TCDD often has an anticarcinogenic effect in vivo (see Section 11.1.1.1.). This anticarcinogenic effect may be the result of increased detoxification or an increased cytotoxicity following increased production of metabolites. 2,3,7,8-TCDD pretreatment has the potential of altering the biological effects of many compounds that are not chemical carcinogens. This modification may reduce the effectiveness, as in the case of zoxazolamine, or increase the effectiveness, as in the case of hexabarbitone (Greig, 1972). The direction and extent of the alteration depends both on the effect of 2,3,7,8-TCDD on the particular enzyme system involved and on whether metabolism is an activating or deactivating process.

# 13. REGULATIONS AND STANDARDS

## 13.1. WATER

Previous release of PCDD-containing herbicides has been one mechanism by which these agents enter the environment. Their high environmental stability and low water solubility (0.2 ppb) make the 2,3,7,8- TCDD tend to settle in the bottom sludge of waterways. The major risk to humans comes from eating bottom-feeding fish in which 2,3,7,8-TCDD has bioaccumulated. The U.S. EPA has set criteria of $1.3\times10^{-7}$, $1.3\times10^{-8}$ or $1.3\times10^{-9}$ μg 2,3,7,8-TCDD/ℓ based on estimated human lifetime cancer risks of $10^{-5}$, $10^{-6}$ and $10^{-7}$, respectively. These criteria are based on the assumption of a daily consumption of 6.5 g contaminated fish and shellfish with the additional daily consumption of 2 ℓ of contaminated drinking water (U.S. EPA, 1984). No information is available regarding concentration limits of 1,2,3,7,8-PeCDD, 1,2,3,7,8,9-HxCDD or 1,2,3,6,7,8- HxCDD in ambient water.

## 13.2. AIR

Many normal combustion processes are suspected of releasing dioxins to the atmosphere. However, the effect on human health from this source is unknown, and no criteria exist regarding concentration limits.

## 13.3. FOOD

According to the FDA (Cordle, 1981, 1983; FDA, 1981, 1983) and the Code of Federal Regulations (41 CFR 321), fish with a 2,3,7,8-TCDD content averaging <25 ppt pose no serious health concern. Federal legal limits for Great Lakes fish distributed in interstate commerce are deemed unnecessary because most of the samples analyzed by the FDA contained <25 ppt. Canada has established a 20 ppt concentration limit for 2,3,7,8-TCDD in Lake Ontario commercial fish imported into the United States to comply with the levels believed by the FDA to be safe (NRCC, 1981a).

A tolerance for hexachlorophene methylenebis (2,3,6-trichlorophenol) in or on feedstock cottenseeds has been established at 0.05 ppm, with the condition that it not contain >0.1 ppm of 2,3,7,8-TCDD (U.S. EPA, 1982c).

No information regarding concentration limits of other dioxin isomers is available.

## 13.4. SUMMARY

The regulation of dioxin by-products in substances such as chlorophenols and 2,4,5-trichlorophenoxyacetic acid is apparently expected to eliminate dioxin releases to the environment. The Canadian concentration limit for 2,3,7,8-TCDD in fish is the only known criterion, and it agrees with levels regarded by the FDA as being protective of human health. In the absence of specific guidelines and standards regarding concentration limits of 2,3,7,8-TCDD, the FDA examines individual contamination situations separately, and gives only general guidance regarding relative risk to humans (Delgado, 1983). No information is available regarding concentration limits for other PCDDs.

# 14. EFFECTS OF MAJOR CONCERN AND HEALTH HAZARD ASSESSMENT

Of the four congeners of PCDDs discussed in this report (2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 1,2,3,7,8,9- and 1,2,3,6,7,8-HxCDD), the majority of toxicologic data are on 2,3,7,8-TCDD. The limited data on the other congeners indicate that they are qualitatively similar in their toxic action to 2,3,7,8-TCDD when comparisons are made in a single species; however, they are less toxic than the 2,3,7,8-TCDD congener. This is illustrated in mice, in which 2,3,7,8-TCDD has an $LD_{50}$ value of 0.88 μmol/kg and 1,2,3,7,8-PeCDD; 1,2,3,6,7,8- and 1,2,3,7,8,9-HxCDD have $LD_{50}$ values of 0.94, 3.19 and 3.67 μmol/kg, respectively (McConnell et al., 1978b). This suggests that either the position or the number of chlorine effects the toxicity of the PCDDs.

In more recent studies using biochemical endpoints, Poland et al. (1979), Bradlaw and Casterline (1979) and Bradlaw et al. (1980) supported the contention that the position and number of chlorines on TCDD, PeCDD and HxCDD are critical for the biologic activity of the compound. In this study, the $ED_{50}$ for the induction of AHH activity in hepatoma cells in culture was used to establish a range of potency for congeners of PCDDs. Although acute toxicity and induction of AHH activity have been used to quantify the difference in the biologic activity of the congeners 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD and 1,2,3,7,8,9-HxCDD, the extrapolation of this data to estimate quantitative dose-response relationships for the chronic toxicity of individual congeners is not sufficiently supported at the present time. From the following data described, it is clear that sufficient information for quantitative hazard assessment is available only for 2,3,7,8-TCDD and a mixture of the two HxCDD congeners.

## 14.1. PRINCIPAL EFFECTS

14.1.1. Toxicity. The principal effect observed in all species after acute exposure to 2,3,7,8-TCDD is weight loss and thymic atrophy (see Table 8-1). The decrease in weight proceeds over a protracted length of time even after a single exposure to a lethal dose. By the time of death, an almost complete absence of body fat stores was often observed. At death, severe deterioration of the animal was observed; however, there was no specific lesion to associate with the cause of death. This was particularly evident in the guinea pig, the most sensitive species to 2,3,7,8-TCDD toxicity. Necropsy revealed no remarkable alteration in any internal organ except for thymic atrophy (Gupta et al., 1973). Although liver damage was observed in rats, rabbits and mice (Schwetz et al., 1973), there are insufficient data to indicate that this effect is the underlying cause of mortality after acute exposure to 2,3,7,8-TCDD. Also, in the guinea pig and monkey, which have the same general progression of gross signs of toxicity as do rats, rabbits and mice, there is only mild liver damage (see Section 8.1.). In addition, 2,3,7,8-TCDD is an immunosuppressant in mice (see Section 8.1.1.4.).

As a result of the long time necessary for the development of toxic symptoms in animals, subchronic and chronic studies are better able to define dose and effect relationships than are acute studies. Subchronic and chronic animal studies that define NOELs and LOELs are summarized in Table 14-1 for orally administered 2,3,7,8-TCDD. The NOEL for subchronic exposure is ~10 times higher than that observed for chronic exposures, suggesting that the cumulative dose might be an important factor in 2,3,7,8-TCDD toxicity. There are only limited data on the NOEL and LOEL for HxCDD

## TABLE 14-1

No-Observed-Effect Levels and Low-Observed-Effect Levels Obtained from Subchronic and Chronic Oral Toxicity Studies of 2,3,7,8-TCDD

| Species/Strain | µg/kg/day | | Duration of Exposure | Duration of Study | Reported Effect | Reference |
|---|---|---|---|---|---|---|
| | NOEL | LOEL | | | | |
| Rat/Sprague-Dawley | 0.01 | 0.1 | 13 weeks | 26 weeks | decreased bw | Kociba et al., 1976 |
| Rat/Osborne-Mendel | 0.07 | 0.14 | 13 weeks | 13 weeks | toxic hepatitis | NTP, 1980a |
| Rat/Sprague-Dawley | 0.0014 | 0.014 | 16 weeks | 40 weeks | elevated porphyrin levels | Goldstein et al., 1982b |
| Rat/Sprague-Dawley | ND | 0.014 | 28 weeks | 40 weeks | fatty changes in the liver, decreased bw | King and Roesler, 1974 |
| Mice/B6C3F1 | ND | 0.014 | 13 weeks | 13 weeks | toxic hepatitis | NTP, 1980a |
| Monkey/Rhesus | ND | <0.02 | 36 weeks | 52 weeks | pancytopenia | Allen et al., 1977 |
| Rat/Sprague-Dawley | 0.001 | 0.01 | 104 weeks | 104 weeks | degenerative and necrotic changes in the liver | Kociba et al., 1978a, 1979 |
| Rat/Osborne-Mendel | 0.0014 | 0.007 | 104 weeks | 107 weeks | toxic hepatitis | NTP, 1980a |
| Mice/Swiss | ND | 0.001 | 52 weeks | life | dermatitis and amyloidosis | Toth et al., 1979 |

ND = Not determined

(Table 14-2) and these were obtained from studies using a 1:2 mixture of 1,2,3,6,7,8- and 1,2,3,7,8,9-HxCDD. As observed with 2,3,7,8-TCDD, there is a suggestion that the cumulative dose of this mixture is an important consideration in defining a NOEL. For both 2,3,7,8-TCDD and the mixture of HxCDD, the liver appeared to be a target organ.

2,3,7,8-TCDD has been shown to produce fetal anomalies in rats, mice, rabbits, ferrets and chickens (see Table 9-2). In mice fetuses, 2,3,7,8-TCDD induces cleft palate and kidney malformations, while in rat fetuses, hemorrhage, edema and a number of anomalies were observed. There was only one study available assessing the teratogenicity of 2,3,7,8-TCDD in rabbits reported by Giavini et al. (1982b) in which increases in extra ribs and total soft-tissue anomalies were observed. In mice, 1 μg/kg/day given for 9-10 days during the middle of gestation was the minimum dose necessary to elicit a teratogenic response (Smith et al., 1976; Moore et al., 1973), while dilated renal pelvis and decreased fetal weight were observed in the rat fetuses of dams receiving doses of 2,3,7,8-TCDD as low as 0.001 μg/kg/day throughout gestation. The statistical and biological significance of effects at this later dose, however, is argued (Murray et al., 1979; Nisbet and Paxton, 1982; U.S. EPA, 1979c). The fetuses of rats appear to be very sensitive to the effects of 2,3,7,8-TCDD, with adverse effects occurring at maternal exposures that were similar to the NOEL observed in chronic studies (see Table 14-1). Also, Schwetz et al. (1973) demonstrated that HxCDD (isomers not specified) was both fetotoxic and teratogenic when administered to pregnant rats at 100 μg/kg on days 6-15 of gestation.

Some epidemiology studies have shown a positive association between exposure to 2,4,5-T, of which 2,3,7,8-TCDD is a known contaminant, and birth

## TABLE 14-2

### No-Observed-Effect Levels and Low-Observed-Effect Levels Obtained from Subchronic and Chronic Oral Toxicity Studies of HxCDD[a,b]

| Species/Strain | µg/kg/day NOEL | µg/kg/day LOEL | Duration of Exposure | Duration of Study | Reported Effects |
|---|---|---|---|---|---|
| Rat/Osborne-Mendel | 0.35 | 0.7 | 13 weeks | 13 weeks | hepatotoxicity |
| Mice/B6C3F1 | 0.7 | 1.4 | 13 weeks | 13 weeks | hepatotoxicity |
| Rat/Osborne-Mendel | ND | 0.18 | 104 weeks | 107 weeks | toxic hepatitis |
| Mice/B6C3F1 | ND | 0.18 | 104 weeks | 107 weeks | toxic hepatitis |

[a] Source: NTP, 1980b

[b] The HxCDD was a 1:2 mixture of 1,2,3,6,7,8- and 1,2,3,7,8,9-HxCDD.

ND = Not determined

defects or abortions. Other studies have failed to demonstrate an association (see Section 9.2.). These studies in humans can neither support nor refute the animal teratogenicity data, since among many other difficulties in interpreting human data the exposures were always mixed, and there were inadequate data concerning the levels of 2,3,7,8-TCDD to which the populations were exposed.

Animal studies also demonstrate that 2,3,7,8-TCDD is a carcinogen (see Table 11-1). The limited studies by Van Miller et al. (1977a,b) and Toth et al. (1978, 1979) indicated that 2,3,7,8-TCDD caused a variety of tumors in rats and mice, and the more intensive studies by Kociba et al. (1978a) and NTP (1980a) support these early findings. Also, papillomas have been reported in female mice after dermal application of 2,3,7,8-TCDD (NTP, 1980b), and using the skin tumorigenesis model, it has been shown that 2,3,7,8-TCDD may affect the carcinogenic potential of other chemical carcinogens (see Section 11.1.1.2.). Human exposure to 2,3,7,8-TCDD has resulted from contamination of other polychlorinated compounds with 2,3,7,8-TCDD (see Section 11.1.3.).

A 1:2 mixture of 1,2,3,6,7,8- and 1,2,3,7,8,9-HxCDD also has been tested for carcinogenicity in rats and mice treated by gavage and by dermal application in mice (NTP, 1980c,d). In both species, this mixture produced liver tumors when administered by gavage, while in the dermal study there was no increase in the incidence of skin tumors.

Epidemiological studies of workers exposed to chemicals contaminated with 2,3,7,8-TCDD such as 2,4,5-trichlorophenoxyacetic acid and 2,4,5-trichlorophenol are consistent with the position that 2,3,7,8-TCDD is probably carcinogenic for humans; the available evidence indicates an excess incidence of soft tissue sarcoma. Because 2,3,7,8-TCDD is almost always found in association with the materials (chlorophenols, combustion products, etc.) it may never be possible to evaluate the carcinogenicity of 2,3,7,8-TCDD by itself in humans.

**14.1.2. Mutagenicity.** There have been many studies of the mutagenic potential of 2,3,7,8-TCDD (see Chapter 10). In vitro assays using bacteria and yeast have generally indicated that 2,3,7,8-TCDD is not a mutagen. These negative results were obtained both in the presence and absence of a mammalian metabolic activation system. A few studies have reported positive results (Hussain et al., 1972; Seiler, 1973; Bronzetti et al., 1980); however, these positive studies had deficiencies in either experimental design, or were reported only qualitatively with inadequate description of experimental detail for evaluation. With the available data, it is impossible to assert whether or not 2,3,7,8-TCDD is devoid of mutagenic potential. There are also some conflicting data from humans and animal studies that indicate that 2,3,7,8-TCDD causes chromosomal aberrations. Because the human data are derived from populations in which exposure to other biologically active compounds is possible, and because the increases observed in animal studies were small, it is still not substantiated that 2,3,7,8-TCDD produces clastogenic changes.

Pertinent data regarding the mutagenic potential of 1,2,3,7,8-PeCDD, 1,2,3,7,8,9-HxCDD or 1,2,3,6,7,8-HxCDD could not be found in the available literature.

## 14.2. SENSITIVE POPULATIONS

Although there are no data from human studies to indicate the presence of sensitive populations, the data from animal studies suggest that the fetus and newborn may be at greater risk. Studies in chickens, rats, mice, rabbits, ferrets and monkeys have shown that in utero exposure to 2,3,7,8-TCDD can result in malformations, fetal toxicity and abortions (see Table 9-2). The lowest dose reported to adversely affect the fetus in utero was 0.001 μg/kg/day administered to the dams throughout gestation (from Murray et al., 1979, according to Nisbet and Paxton, 1982); this dose is similar to the NOEL reported for chronic exposure of adult rats (see Table 14-1). Moore et al. (1973) observed that the nursing of pups on mothers exposed to 2,3,7,8-TCDD could also result in kidney anomalies detected at the time of weaning. These data suggest that both the fetus and the newborn may be more sensitive than the adult to the adverse effects of exposure to 2,3,7,8-TCDD.

In addition, 2,3,7,8-TCDD is known to be a powerful inducer of the MFO system. There is information to indicate that MFO induction by 2,3,7,8-TCDD can affect the biologic activity of other xenobiotics that require metabolic activation (see Chapter 12). Scarpelli et al. (1980), for example, demonstrated that pretreatment of hamsters with 2,3,7,8-TCDD resulted in greater activation of mutagenic nitrosamines when assayed in vitro with isolated microsomes. Individuals exposed to chemicals that are activated by the MFO may experience a synergistic effect and be at greater risk. In a similar manner, if the MFO detoxifies a xenobiotic, pretreatment with 2,3,7,8-TCDD may antagonize the action of other compounds.

## 14.3. FACTORS INFLUENCING HEALTH HAZARD ASSESSMENT

It is expected that the PCDDs discussed here would be highly persistent compounds in the environment, and that human exposure may occur through ingestion of contaminated food and water, by inhalation of the compound absorbed to respirable particulates, or through dermal contact. Although potential exposure may occur by all routes, most of the toxicologic information is from studies of oral exposure. The limited observation of toxic effects in humans and animals after dermal contact with 2,3,7,8-TCDD in organic solvents indicates that dermal absorption occurs. Poiger and Schlatter (1980) have shown in rats that both dermal and GI absorption is dependent on the vehicle. Greatest absorption after oral exposure occurred when 2,3,7,8-TCDD was administered in organic solvent followed by aqueous suspension, with little absorption occurring if the 2,3,7,8-TCDD was adsorbed onto activated carbon. In a similar manner, dermal absorption was poor if the 2,3,7,8-TCDD was applied in a soil and water paste. Inhalation exposure is likely to occur through airborne particulate matter containing absorbed 2,3,7,8-TCDD; however, it is not possible with the available data to predict how efficiently absorption will occur through the respiratory tract. The use of standard respiratory absorption assumptions in risk assessment are most likely to provide conservative criteria levels.

## 14.4. QUALITATIVE HEALTH HAZARD ASSESSMENT

The data available from animal studies are sufficient to provide some assessment of the human health hazards associated with exposure to 2,3,7,8-TCDD and a mixture of 1,2,3,7,8,9- and 1,2,3,6,7,8-HxCDD. The only data available on 1,2,3,7,8-PeCDD are an acute $LD_{50}$ value and studies of induction of AHH activity. Although both types of data indicate that 1,2,3,7,8-PeCDD might have slightly less biological activity than 2,3,7,8-TCDD, the data are insufficient to adequately predict the risk associated with a particular dose of 1,2,3,7,8-PeCDD. This would be the case if attempts were made to use these data from acute exposure to extrapolate the effects of chronic exposure whether these effects are toxic or carcinogenic. For the other PCDDs discussed, the hazard assessment can be based on toxicity, teratogenicity or carcinogenicity.

Although there have been human epidemiology studies investigating the toxic, reproductive and carcinogenic effect of exposure to 2,3,7,8-TCDD, these studies have major deficiencies for use in health assessment. 2,3,7,8-TCDD is a contaminant of the chemicals 2,4,5-T and TCP, and all human data are derived from populations exposed to mixtures. In these studies, it is not possible to attribute with certainty any observed effect to exposure to 2,3,7,8-TCDD. Also, exposure data of sufficient quality are not available to define a dose-response relationship in human population. Without adequate exposure data, health assessments cannot be made.

14.4.1. Animal Toxicity Data. Animal studies that are useful for hazard assessment are studies with adequate experimental design to define the levels of exposure that produce threshold effects. Tables 14-1 and 14-2 summarize these studies, providing data on NOEL (or NOAEL) and LOEL (or LOAEL). Since there is suggestive evidence that the cumulative dose is important to the toxicity of 2,3,7,8-TCDD and the mixture of HxCDD tested, the chronic toxicity studies would be more appropriately used for hazard assessment. The NOEL from the two studies in rats (Kociba et al., 1978a, 1979; NTP, 1980a) are 0.001 and 0.0014 μg/kg/day; however, in the mouse (NTP, 1980a), the dose of 0.07 μg/kg/day was a FEL, as indicated by fatty changes in the liver, and 0.007 was a NOEL.

In addition, it may be inappropriate to derive a toxicity-based hazard assessment for 2,3,7,8-TCDD from these chronic studies, since a 3-generation study by Murray et al. (1979) indicates that exposure of pregnant rats to this dose of 2,3,7,8-TCDD (0.001 μg/kg/day) throughout gestation resulted in the observation of dilated renal pelvis in the fetuses. Murray et al. (1979) and U.S. EPA (1979c) consider this effect not to be treatment-related because it occurred in only one generation at this dose and not at higher doses. Hence, 0.001 μg/kg/day represented a NOAEL. However, a reevaluation of these data by different statistical methods (Nisbet and Paxton, 1982) indicated a statistically significant increase of dilated renal pelvis at higher doses, as well as the lowest one, and lower fetal weight in the 0.001 μg/kg group. With these data, 0.001 μg/kg could be considered a LOAEL. No other studies are available regarding the effects of 2,3,7,8-TCDD at even lower doses.

A toxicity-based hazard assessment is also possible for the mixture of HxCDD tested by NTP (1980b). As is shown in Table 14-2, however, the description of the histologic observations was not sufficiently detailed to determine whether the low dose represented a NOAEL or a LOAEL. These data could be used for hazard assessment in either case with an additional uncertainty factor for a LOAEL (Federal Register, 1980b).

14.4.2. Animal Carcinogenicity. In addition to the inadequate data base for a toxicity-based hazard assessment, the strong evidence of carcinogenicity in animals for 2,3,7,8-TCDD would justify a carcinogenicity-based assessment. That two adequate cancer bioassays used sufficiently large groups of animals exposed for an appreciable portion of their lifespan indicates that 2,3,7,8-TCDD is an animal carcinogen (NTP, 1980a; Kociba et al., 1978a) (Table 14-3). In the NTP (1980a) study, male rats developed follicular-cell adenomas or carcinomas of the thyroid. Female rats and mice of both sexes had increased incidences of follicular-cell adenomas of the thyroid. In the study by Kociba et al. (1978a), rats maintained on diets that provided doses of 0.0, 0.001, 0.01 and 0.1 μg/kg/day had elevated incidences of carcinomas of the hard palate and tongue, and adenoma of the adrenal cortex in males of the high dose group, and carcinomas of the liver, tongue and lungs in females of the high-dose group. The evidence is sufficient to indicate that 2,3,7,8-TCDD is an animal carcinogen.

## TABLE 14-3

### Carcinogenicity Bioassays of 2,3,7,8-TCDD

| Exposure Route | Species/Strain | Sex | Dose or Exposure | Duration of Treatment | Duration of Study | Vehicle | Tumor Type | Tumor Incidence | Reference |
|---|---|---|---|---|---|---|---|---|---|
| Gavage | rats/ Osborne-Mendel | M | 0.0 μg/kg/week | 104 weeks | 105 weeks | corn oil-acetone (9:1) | follicular-cell adenomas or carcinoma of the thyroid | 1/69 | NTP, 1980a |
| | | | 0.01 μg/kg/week | 104 weeks | 107 weeks | corn oil-acetone (9:1) | follicular-cell adenomas or carcinoma of the thyroid | 5/48 | |
| | | | 0.05 μg/kg/week | 104 weeks | 107 weeks | corn oil-acetone (9:1) | follicular-cell adenomas or carcinoma of the thyroid | 8/50 | |
| | | | 0.5 μg/kg/week | 104 weeks | 107 weeks | corn oil-acetone (9:1) | follicular-cell adenomas or carcinoma of the thyroid | 11/50 | |
| Gavage | rats/ Osborne-Mendel | F | 0.0 μg/kg/week | 104 weeks | 105 weeks | corn oil-acetone (9:1) | neoplastic nodule or hepatocellular carcinoma of the liver | 5/75 | NTP, 1980a |
| | | | 0.1 μg/kg/week | 104 weeks | 107 weeks | corn oil-acetone (9:1) | neoplastic nodule or hepatocellular carcinoma of the liver | 1/49 | |
| | | | 0.05 μg/kg/week | 104 weeks | 107 weeks | corn oil-acetone (9:1) | neoplastic nodule or hepatocellular carcinoma of the liver | 3/50 | |
| | | | 0.5 μg/kg/week | 104 weeks | 107 weeks | corn oil-acetone (9:1) | neoplastic nodule or hepatocellular carcinoma of the liver | 14/49 | |

TABLE 14-3 (cont.)

| Exposure Route | Species/Strain | Sex | Dose or Exposure | Duration of Treatment | Duration of Study | Vehicle | Tumor Type | Tumor Incidence | Reference |
|---|---|---|---|---|---|---|---|---|---|
| Gavage | mice/B6C3F1 | M | 0.0 μg/kg/week | 104 weeks | 105 weeks | corn oil-acetone (9:1) | hepatocellular carcinoma | 8/73 | NTP, 1980a |
| | | | 0.01 μg/kg/week | 104 weeks | 107 weeks | corn oil-acetone (9:1) | hepatocellular carcinoma | 9/49 | |
| | | | 0.05 μg/kg/week | 104 weeks | 107 weeks | corn oil-acetone (9:1) | hepatocellular carcinoma | 8/49 | |
| | | | 0.5 μg/kg/week | 104 weeks | 107 weeks | corn oil-acetone (9:1) | hepatocellular carcinoma | 17/50 | |
| Gavage | mice/B6C3F1 | F | 0.0 μg/kg/week | 104 weeks | 105 weeks | corn oil-acetone (9:1) | hepatocellular carcinoma,<br>follicular-cell adenomas of the thyroid | 1/73<br>0/69 | NTP, 1980a |
| | | | 0.04 μg/kg/week | 104 weeks | 107 weeks | corn oil-acetone (9:1) | hepatocellular carcinoma,<br>follicular-cell adenomas of the thyroid | 2/50<br>3/50 | |
| | | | 0.2 μg/kg/week | 104 weeks | 107 weeks | corn oil-acetone (9:1) | hepatocellular carcinoma,<br>follicular-cell adenomas of the thyroid | 2/48<br>1/47 | |
| | | | 2.0 μg/kg/week | 104 weeks | 107 weeks | corn oil-acetone (9:1) | hepatocellular carcinoma,<br>follicular-cell adenomas of the thyroid | 6/47<br>5/46 | |
| Oral | rat/ Sprague-Dawley | M | 0.0 μg/kg/day | 105 weeks | 105 weeks | in diet | squamous cell carcinoma of the hard palate,<br>squamous cell carcinoma of the tongue,<br>adenoma of the adrenal cortex | 0/85<br>0/85<br>0/85 | Kociba et al., 1978a |

TABLE 14-3 (cont.)

| Exposure Route | Species/Strain | Sex | Dose or Exposure | Duration of Treatment | Duration of Study | Vehicle | Tumor Type | Tumor Incidence | Reference |
|---|---|---|---|---|---|---|---|---|---|
| Oral (cont.) | rat/ Sprague-Dawley | | 0.001 µg/kg/day | 105 weeks | 105 weeks | in diet | squamous cell carcinoma of the hard palate, | 0/50 | Kociba et al., 1978a |
| | | | | | | | squamous cell carcinoma of the tongue, | 1/50 | |
| | | | | | | | adenoma of the adrenal cortex | 0/50 | |
| | | | 0.01 µg/kg/day | 105 weeks | 105 weeks | in diet | squamous cell carcinoma of the hard palate, | 0/50 | |
| | | | | | | | squamous cell carcinoma of the tongue, | 1/50 | |
| | | | | | | | adenoma of the adrenal cortex | 2/50 | |
| | | | 0.1 µg/kg/day | 105 weeks | 105 weeks | in diet | squamous cell carcinoma of the hard palate, | 4/50 | |
| | | | | | | | squamous cell carcinoma of the tongue, | 3/50 | |
| | | | | | | | adenoma of the adrenal cortex | 5/50 | |
| Oral | rat/ Sprague-Dawley | F | 0.0 µg/kg/day | 105 weeks | 105 weeks | in diet | hepatocellular carcinoma, | 1/86 | Kociba et al., 1978a |
| | | | | | | | squamous cell carcinoma of the hard palate, | 0/86 | |
| | | | | | | | squamous cell carcinoma of the lung | 0/86 | |
| | | | 0.001 µg/kg/day | 105 weeks | 105 weeks | in diet | hepatocellular carcinoma, | 0/50 | |
| | | | | | | | squamous cell carcinoma of the hard palate, | 0/50 | |
| | | | | | | | squamous cell carcinoma of the lung | 0/50 | |
| | | | 0.01 µg/kg/day | 105 weeks | 105 weeks | in diet | hepatocellular carcinoma, | 2/50 | |
| | | | | | | | squamous cell carcinoma of the hard palate, | 1/50 | |
| | | | | | | | squamous cell carcinoma of the lung | 0/50 | |
| | | | 0.1 µg/kg/day | 105 weeks | 105 weeks | in diet | hepatocellular carcinoma, | 11/49 | |
| | | | | | | | squamous cell carcinoma of the hard palate, | 4/49 | |
| | | | | | | | squamous cell carcinoma of the lung | 7/49 | |

A single bioassay tested a mixture of the two congeners of HxCDD for carcinogenicity (NTP, 1980b). The results summarized in Table 14-4 show that male and female rats and mice exposed to this mixture of HxCDD had increased incidences of neoplastic nodules or carcinomas of the liver. Increased incidence of tumors in two species is sufficient to indicate that this mixture was carcinogenic to animals; however, caution is required in interpreting these data for hazard evaluation since the NTP (1980a) study used a mixture containing two isomers, 1,2,3,6,7,8- and 1,2,3,7,8,9-, of HxCDD and the HxCDD mixture used for this bioassay was found to be contaminated with other PCDDs including 0.09% (±0.03%) of TCDD. The specific isomer of PCDDs was not identified. There is insufficient evidence to confirm whether both isomers are independently carcinogenic or whether only one isomer or this specific mixture is needed to elicit a carcinogenic response. Since the position of the chlorines may be extremely important for the toxic/carcinogenic properties of HxCDD, information obtained from this combined exposure may not be applicable to the individual congeners.

TABLE 14-4

Carcinogenicity Bioassays of a 1:2 Mixture of 1,2,3,6,7,8- and 1,2,3,7,8,9-HxCDD

| Exposure Route | Species/Strain | Sex | Dose or Exposure | Duration of Treatment | Duration of Study | Vehicle | Tumor Type | Tumor Incidence | Reference |
|---|---|---|---|---|---|---|---|---|---|
| Gavage | rats/ Osborne-Mendel | M | 0.0 μg/kg/week | 104 weeks | 105 weeks | corn oil-acetone (9:1) | liver neoplastic nodules or hepatocellular carcinoma | 0/74 | NTP, 1980b |
| Gavage | rats/ Osborne-Mendel | M | 1.25 μg/kg/week | 104 weeks | 107 weeks | corn oil-acetone (9:1) | liver neoplastic nodules or hepatocellular carcinoma | 0/49 | NTP, 1980d |
|  |  |  | 2.5 μg/kg/week | 104 weeks | 107 weeks | corn oil-acetone (9:1) | liver neoplastic nodules or hepatocellular carcinoma | 1/50 |  |
|  |  |  | 5.0 μg/kg/week | 104 weeks | 107 weeks | corn oil-acetone (9:1) | liver neoplastic nodules or hepatocellular carcinoma | 4/48 |  |
| Gavage | rats/ Osborne-Mendel | F | 0.0 μg/kg/week | 104 weeks | 105 weeks | corn oil-acetone (9:1) | liver neoplastic nodules or hepatocellular carcinoma | 5/75 | NTP, 1980d |
|  |  |  | 1.25 μg/kg/week | 104 weeks | 107 weeks | corn oil-acetone (9:1) | liver neoplastic nodules or hepatocellular carcinoma | 10/50 |  |
|  |  |  | 2.5 μg/kg/week | 104 weeks | 107 weeks | corn oil-acetone (9:1) | liver neoplastic nodules or hepatocellular carcinoma | 12/50 |  |
|  |  |  | 5.0 μg/kg/week | 104 weeks | 107 weeks | corn oil-acetone (9:1) | liver neoplastic nodules or hepatocellular carcinoma | 30/50 |  |

TABLE 14-4 (cont.)

| Exposure Route | Species/Strain | Sex | Dose or Exposure | Duration of Treatment | Duration of Study | Vehicle | Tumor Type | Tumor Incidence | Reference |
|---|---|---|---|---|---|---|---|---|---|
| Gavage | rats/ Osborne-Mendel | F | 0.0 μg/kg/week | 104 weeks | 105 weeks | corn oil-acetone (9:1) | hepatocellular adenomas or carcinomas | 15/73 | NTP, 1980d |
| | | | 1.25 μg/kg/week | 104 weeks | 108 weeks | corn oil-acetone (9:1) | hepatocellular adenomas or carcinomas | 14/50 | |
| | | | 2.5 μg/kg/week | 104 weeks | 107 weeks | corn oil-acetone (9:1) | hepatocellular adenomas or carcinomas | 14/49 | |
| | | | 5.0 μg/kg/week | 104 weeks | 108 weeks | corn oil-acetone (9:1) | hepatocellular adenomas or carcinomas | 24/48 | |
| Gavage | mice/B6C3F1 | F | 0.0 μg/kg/week | 104 weeks | 106 weeks | corn oil-acetone (9:1) | hepatocellular adenomas or carcinomas | 3/75 | NTP, 1980d |
| | | | 2.5 μg/kg/week | 104 weeks | 108 weeks | corn oil-acetone (9:1) | hepatocellular adenomas or carcinomas | 4/48 | |
| | | | 5.0 μg/kg/week | 104 weeks | 108 weeks | corn oil-acetone (9:1) | hepatocellular adenomas or carcinomas | 6/47 | |
| | | | 10.0 μg/kg/week | 104 weeks | 107 weeks | corn oil-acetone (9:1) | hepatocellular adenomas or carcinomas | 10/47 | |

# 15. REFERENCES

Abernethy, D.J., W.F. Greenlee, J.C. Huband and C.J. Boreiko. 1985. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) promotes the transformation of C3H/10T$_{1/2}$ cells. Carcinogenesis. 6: 651-653.

ACP (Advisory Committee on Pesticides). 1980. Further Review of the Safety for Use in the U.K. of the Herbicide 2,4,5-T, Pesticides Branch, Ministry of Agriculture, Fisheries and Food, London, England.

Adamoli, P., E. Angeli, G. Bandi, et al. 1978. Analysis of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the Seveso area. In: Chlorinated Phenoxy Acids and Their Dioxins, C. Rainel, Ed. Ecol. Bull. 27: 31-38.

Agarwal, A.K., H.A. Tilson and S.C. Bondy. 1981. 3,4,3',4'-Tetrachlorobiphenyl given to mice prenatally produces long-term decreases in striatal dopamine and receptor binding sites in the caudate nucleus. Toxiol. Lett. 7: 417-424.

Aitio, A. and M.G. Parkki. 1978. Organ specific induction of drug metabolizing enzymes by 2,3,7,8-tetrachlorodibenzo-p-dioxin in the rat. Toxicol. Appl. Pharmacol. 44(1): 107-114.

Albro, P.W. 1979. Problems in analytical methodology: Sample handling, extraction, and clean up. Ann. NY Acad. Sci. 320: 19-27.

Albro, P.W. and B.J. Corbett. 1977. Extraction and clean up of animal tissues for subsequent determination of mixtures of chlorinated dibenzo-p-dioxin and dibenzofurans. Chemosphere. 6: 381-385.

Albro, P.W., J.T. Corbett, M. Harriss and L.D. Lawson. 1978. Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on lipid profiles in tissue of the Fischer rat. Chem. Biol. Interact. 23(3): 315-330.

Albro, P.W., M.I. Luster, K. Chae, et al. 1979. A radioimmunoassay for chlorinated dibenzo-p-dioxins. Environ. Biol. Chem. Branch, Natl. Inst., Environ. Health Sci., Research Triangle Park, NC 27709.

Aldred, J.E. 1978. Report of the Consultative Council on Congenital Abnormalities in the Yarrom District. Minister of Health, Melbourne, Victoria, Australia. (Cited in Milby et al., 1980)

Allen, J.R. and L.A. Carstens. 1967. Light and electron microscopic observations in Macaca mulatta monkeys fed toxic fat. Am. J. Vet. Res. 28: 1613-1629.

Allen, J.R. and J.J. Lalich. 1962. Response of chickens to prolonged feeding of crude "toxic fat." Proc. Sox. Exp. Biol. Med. 109: 48-51.

Allen, J.R., J.P. Van Miller and D.H. Norback. 1975. Tissue distribution, excretion, and biological effects of (14-c) tetrachlorodibenzo-p-dioxin in rats. Food Cosmet. Toxicol. 13(5): 501-505.

Allen, J.R., D.A. Barsotti, J.P. Van Miller, L.J. Abrahamson and J.J. Lalich. 1977. Morphological changes in monkeys consuming a diet containing low levels of TCDD. Food Cosmet. Toxicol. 15: 401.

Allen, J.R., D.A. Barsotti, L.K. Lambrecht and J.P. Van Miller. 1979. Reproductive effects of halogenated aromatic hydrocarbons on nonhuman primates. Ann. NY Acad. Sci. 320: 419-425.

Anderson, E.L. and the Carcinogen Assessment Group of the U.S. Environmental Protection Agency. 1983. Quantitative approaches in use to assess cancer risk. Risk Analysis. 3(4): 277.

Anderson, H.A., R. Lilis and I.J. Selikoff. 1978. Unanticipated prevalence of symptoms among dairy farmers in Michigan and Wisconsin. Environ. Health Perspect. 23: 217-226.

Anonymous. 1979. Dioxin is found in cleanup worker's blood. Chemical Week. 124: 22.

Anonymous. 1982. Phenoxyherbicides, trichlorophenols, and soft-tissue sarcomas. May 8. Lancet. p. 1051-1052.

Appelgren, L.-E., I. Brandt, E.B. Brittebo, M. Gillner and J.-A. Gustafsson. 1983. Autoradiography of 2,3,7,8-tetrachloro[$^{14}$C]-dibenzo-p-dioxin (TCDD): Accumulation in the nasal mucosa. Chemosphere. 12(4/5): 545-548.

Arkansas Department of Pollution Control and Ecology. 1983. Summary of Technical Data. Jacksonville, AR.

AWPI (American Wood Preservers Institute). 1977. Memo to the Office of Pesticide Program, U.S. EPA, Research Triangle Park, NC.

Ax, R.L. and L.G. Hansen. 1975. Effects of purified PCB analogs on chicken reproduction. Poult. Sci. 54: 895-900.

Axelson, O. 1980. A note on observational bias case-referent studies. Scand. J. Work Environ. Health. 6(1): 80-82.

Axelson, O., L. Sundell, K. Anderson, C. Edling, C. Hogstedt and H. Kling. 1980. Herbicide exposure and tumor mortality: An updated epidemiologic investigation on Swedish railroad workers. Scand. J. Work Environ. Health. 6: 73-79.

Baars, A.J., M. Jansen and D.D. Breimer. 1978. The influence of phenobarbital, 3-methylcholanthrene, and 2,3,7,8-tetrachlorodibenzo-p-dioxin on glutathione S-transferase activity of rat liver cytosol. Biochem. Pharmacol. 27(21): 2487-2494.

Ball, L.M. and R.S. Chhabra. 1981. Intestinal absorption of nutrients in rats treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). J. Toxicol. Environ. Health. 8/4: 629-638.

Ballschmiter, K., W. Krämer, H. Magg, et al. 1984. Distribution of polychlorinated dibenzo-dioxin and -furan emissions between particulates, flue gas condensate and impinger absorption in stack gas sampling. Chemosphere. 13: 1139-1142.

Bandiera, S., T.W. Sawyer, M.A. Campbell, P. Fujita and S. Safe. 1983. Comparative binding to the cytosolic 2,3,7,8-tetrachlorodibenzo-p-dioxin receptor: Effects of structure on the affinities of substituted halogenated biphenyls -- a QSAR analysis. Biochem. Pharmacol. 32: 3803-3813.

Barnes, D.G. 1983. "Dioxin" Production from Combustion of Biomass and Waste. Presented at the Symposium on Energy from Biomass and Wastes. VII. Lake Buena Vista, FL. Jan. 24-28.

Barsotti, D.A., L.J. Abrahamson and J.R. Allen. 1979. Hormonal alterations in female rhesus monkeys fed a diet containing 2,3,7,8-tetrachlorodibenzo-p-dioxin. Bull. Environ. Contam. Toxicol. 21: 463-469.

Bartsch, H., L. Tomatis and C. Malaveille. 1982. Qualitative and quantitative comparisons between mutagenic activities of chemicals. In: Mutagenicity: New Horizons in Genetic Toxicology, J.A. Heddle, Ed. Academic Press, NY. p. 36-72.

Baughman, R. and M. Meselson. 1973. An analytical method for detecting TCDD (dioxin): Levels of TCDD in samples from Vietnam. Environ. Health Perspect. 5: 27-35.

Beale, M.G., W.T. Shearer, M.M. Karl and A.M. Robson. 1977. Long-term effects of dioxin exposure. Lancet. April 2. p. 748.

Beatty, P.W. and R.A. Neal. 1975. Effect of alteration of hepatic mixed function oxidase activity on the toxicity of 2,3,7,8-tetrachlorodibenzodioxin (TCDD) in rats. Toxicol. Appl. Pharmacol. 33: 151.

Beatty, P. and R.A. Neal. 1976a. Induction of DT-diaphorase by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Biochem. Biophys. Res. Commun. 12. 68(1): 197-204.

Beatty, P.W. and R.A. Neal. 1976b. Induction of DT-diaphorase activity of rat liver by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol. Appl. Pharmacol. 37: 189.

Beatty, P. and R.A. Neal. 1978. Factors affecting the induction of DT-diaphorase by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Biochem. Pharmacol. 27(4): 505-510.

Beatty, P.W., K.J. Lembach, M.A. Holscher and R.A. Neal. 1975. Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on mammalian cells in tissue cultures. Toxicol. Appl. Pharmacol. 31(2): 309-312.

Beatty, P.W., M.A. Holscher and R.A. Neal. 1976. Toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in larval and adult forms of Rana catesbeiana. Bull. Environ. Contam. Toxicol. 16(5): 578-581.

Beatty, P.W., W.K. Vaughn and R.A. Neal. 1978. Effect of alteration of rat hepatic mixed-function oxidase (MFO) activity on the toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Toxicol. Appl. Pharmacol. 45(2): 513-519.

Benedict, W.F., N. Considine and D.W. Nebert. 1973. Genetic differences in aryl hydrocarbon hydroxylase induction and benzo[a]pyrene-produced tumorigenesis in the mouse. Mol. Pharmacol. 9: 266-277.

Benfenati, E., F. Gizzi, R. Reginate, R. Fanelli, M. Lodi and R. Taghaferri. 1983. Polychlorinated dibenzo-p-dioxins (TCDD) and polychlorinated dibenzofurans (PCDF) in emissions from an urban incinerator. II. Correlation between concentration of micropollutants and combustion conditions. Chemosphere. (In press)

Berry, D.L., J. DiGiovanni, M.R. Juchau, W.M. Bracken, G.L. Gleason and T.J. Slaga. 1978. Lack of tumor-promoting ability of certain environmental chemicals in a two-stage mouse skin tumorigenesis assay. 20(1): 101-108.

Berry, D.L., T.J. Slaga, J. DiGiovanni and M.R. Juchau. 1979. Studies with chlorinated dibenzo-p-dioxins, polybrominated biphenyls, and polychlorinated biphenyls in a two-stage system of mouse skin tumorigenesis: Potent anticarcinogenic effects. Ann. NY Acad. Sci. 320: 405-414.

Biocca, M., B.W. Gupta, K. Chae, J.D. McKinney and J.A. Moore. 1981. Toxicity of selected symmetrical hexachlorobiphenyl isomers in the mouse. Toxicol. Appl. Pharmacol. 58: 461-474.

Bisanti, L., F. Bonetti, F. Caramaschi, et al. 1980. Experiences from the accident of Seveso. Acta. Morphol. Acad. Sci. Hung. 28(1-2): 139-157.

Bishop, C.M. and A.H. Jones. 1981. Non-Hodgkin's lymphoma of the scalp in workers exposed to dioxins. Lancet. 2(8242): 369.

Bleiburg, J., M. Wallen, R. Brodkin and I. Applebaum. 1964. Industrially acquired porphyria. Arch. Dermatol. 84: 793-797.

Boeri, E. 1978. Report to Lombardy Regional Authority (unpublished). (Cited in Pocchiari et al., 1979)

Bogen, G. 1979. Symptoms in Vietnam veterans exposed to Agent Orange. J. Am. Med. Assoc. 242(22): 2391.

Bollen, W.B. and L.A. Norris. 1979. Influence of 2,3,7,8-tetrachlorodibenzo-p-dioxin on respiration in a forest floor and soil. Bull. Environ. Contam. Toxicol. 22(4-5): 648-652.

Bonaccorsi, A., R. Fanelli and G. Tognoni. 1978. In the wake of Seveso. Ambio. 7(5-6): 234-239.

Bonaccorsi, A., A. diDomenico, R. Fanelli, et al. 1983. The influence of soil particle adsorption on TCDD biological uptake in the rabbit. Arch. Toxicol. (In press)

Botre, C., A. Memoli and F. Alhaique. 1978. TCDD solubilization and photodecomposition in aqueous solutions. Environ. Sci. Technol. 12(3): 335-336.

Bradlaw, J.A. and J.L. Casterline, Jr. 1979. Induction of enzyme activity in cell culture: A rapid screen for detection of planar polychlorinated organic compounds. J. Assoc. Off. Anal. Chem. 62: 904-906.

Bradlaw, J.A., L.H. Garthoff, D. Graff and N.E. Hurley. 1975. Detection of chlorinated dioxins induction of aryl hydrocarbon hydroxylase activity in rat hepatoma cell culture. Toxicol. Appl. Pharmacol. 33(1): 166.

Bradlaw, J.A., L.H. Garthoff, N.E. Hurley and D. Firestone. 1976. Aryl hydrocarbon hydroxylase activity of twenty-three halogenated dibenzo-p-dioxins (Meeting Abstract). Toxicol. Appl. Pharmacol. 37(1): 119.

Bradlaw, J.A., L.H. Garthoff, N.E. Hurley and D. Firestone. 1980. Comparative induction of aryl hydrocarbon hydroxylase activity _in vitro_ by analogues of dibenzo-p-dioxin. Food Cosmet. Toxicol. 18: 627-635.

Brewster, D.W., B.V. Madhukar and F. Matsumura. 1982. Influence of 2,3,7,8-TCDD on the protein composition of the plasma membrane of hepatic cells from the rat. Biochem. Biophys. Res. Commun. 107(1): 68-74.

Bronzetti, G., I. Lee, E. Zeiger, et al. 1980. Genetic effects of TCDD _in vitro_ and _in vivo_ using D7 strain of _S_. _cerevisiae_. Mutat. Res. 74/3 (75).

Bronzetti, G., C. Bauer, C. Corsi, R. Del Carratare, R. Neeri and M. Paoline. 1983. Mutagenicity study of TCDD and ashes from urban incinerator "_in vitro_" and "_in vivo_" using yeast D7 strain. Chemosphere. 12: 549-553.

Brumley, W.C., J.A.G. Roach, J.A. Sphon, et al. 1981. Low-resolution multiple ion detection gas chromatographic-mass spectrometric comparison of six extraction-cleanup methods for determining 2,3,7,8-tetrachlorodibenzo-p-dioxin in fish. J. Agric. Food Chem. 29(5): 1040-1046.

Bumb, R.R., W.B. Crummet, S.S. Cutie, et al. 1980. Trace chemistries of fire: A source of chlorinated dioxins. Science. 210: 385-390.

Burns, L.H., D.M. Cline and R.R. Lassiter. 1981. Exposure analysis modeling system (EXAMS). Prepared by the Environmental Research Laboratory, ORD, U.S. EPA, Athens, GA.

Bus, J.S. and J.E. Gibson. 1979. Lipid peroxidation and its role in toxicology. In: Reviews in Biochemical Toxicology, E. Hodgson, J.R. Bend and R.M. Philpot, Ed. Elsevier/North Holland, NY. 1: 125-149.

Buser, H.R. 1975. Polychlorinated dibenzo-p-dioxin: Separation and identification of isomers by gas chromatography-mass spectrometry. J. Chromatogr. 114: 95-108.

Buser, H.R. 1976. High-resolution gas chromatography of polychlorinated dibenzo-p-dioxins and dibenzofurans. Anal. Chem. 48: 1553-1557.

Buser, H.R. 1978. Analysis of TCDD by gas chromatography-mass spectrometry using glass capillary columns. In: Dioxin: Toxicological and Chemical Aspects, F. Cattabeni, A. Cavallaro and G. Galli, Ed. SP Medical and Scientific Books, NY. p. 27-41.

Buser, H.R. 1979. Formation of polychlorinated dibenzofurans (PCDFs) and dibenzo-p-dioxins (PCDDs) from pyrolysis of chlorobenzenes. Chemosphere. 6: 415-424.

Buser, H.R. and H.P. Bosshardt. 1976. Determination of polychlorinated dibenzo-p-dioxins and dibenzofurans in commercial pentachlorophenols by combined GC-MS. J. Assoc. Off. Anal. Chem. 59: 562-569.

Buser, H.R. and H.P. Bosshardt. 1978. Polychlorinated dibenzo-p-dioxins, dibenzofurans, and benzenes in the fly ash of municipal and industrial incinerators. Mitt. Geb. Lebens. Hyg. 69: 191-199.

Buser, H.R. and C. Rappe. 1978. Identification of substitution patterns in polychlorinated dibenzo-p-dioxins (PCDDs) by mass spectrometry. Chemosphere. 7: 199-211.

Buser, H.R. and C. Rappe. 1980. High resolution gas chromatography of the 22 tetrachlorodibenzo-p-dioxin isomers. Anal. Chem. 52: 2257-2262.

Buser, H.R. and C. Rappe. 1983. Isomer-specific separation and analysis of 2,3,7,8-substituted polychlorinated dibenzo-p-dioxins (PCDDs) using high-resolution gas chromatography and mass spectrometry. Anal. Chem. (In review)

Buser, H.R., H.P. Bosshardt and C. Rappe. 1978. Identification of polychlorinated dibenzo-p-dioxin isomers formed in fly ash. Chemosphere. 7: 165-172.

Buu-Hoi, N.P., Pham-Huu-Chanh, G. Sesque, M.C. Azum-Gelade and G. Saint-Ruf. 1972. Organs as targets of dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin) intoxication. Naturwissenschaften. 59(4): 174-175.

Callahan, M.A., M.W. Slimak, N.W. Gabel, et al. 1979. Water-Related Environmental Fate of 129 Priority Pollutants. Vol. I. EPA 440/4-79-029a, Office of Water Planning and Standards, Office of Water and Wastewater Management, U.S. EPA, Washington, DC.

Camoni, I., A. DiMuccio, D. Pontecorvo, et al. 1983. Lack of in vitro oxidation of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in the presence of laccase from Polyporus versicolor fungus. Chemosphere. (In press)

Cantoni, L., M. Rizzardini, G. Belvedere, R. Cantoni, R. Fanelli and M. Salmona. 1981. Induction of mixed-function oxidase by chronic treatment with 2,3,7,8-tetrachlorodibenzo-p-dioxin in female rats. Toxicology. 21(2): 159-167.

Caramaschi, F., G. del Crono, C. Favarett, S.E. Giambelluca, E. Montesarchio and G.M. Fara. 1981. Chloracne following environmental contamination by TCDD in Seveso, Italy. Int. J. Epidemiol. 10(2): P135-143.

Carlstedt-Duke, J.M. 1979. Tissue distribution of the receptor for 2,3,7,8-tetrachlorodibenzo-p-dioxin in the rat. Cancer Res. 39(8): 3172-3176.

Carlstedt-Duke, J.M., G. Elfstrom, B. Hogberg and J.A. Gustafsson. 1979. Ontogeny of the rat hepatic receptor for 2,3,7,8-tetrachlorodibenzo-p-dioxin and its endocine independence. Cancer Res. 39(11): 4653-4656.

Carlstedt-Duke, J.M.B., V.B. Harnemo, B. Hogberg and J.A. Gustafsson. 1981. Interaction of the hepatic receptor protein for 2,3,7,8-tetrachlorodibenzo-p-dioxin with DNA. J. Biochim. Biophys. Acta. 672(2): 131-141.

Carter, C.D., R.D. Kimbrough, J.A. Liddle, et al. 1975. Tetrachlorodibenzo-p-dioxin: An accidental poisoning episode in horse arenas. Science. 188: 738-740.

Cavallaro, A., G. Bartolozzi, D. Carreri, G. Bandi, L. Luciani and G. Villa. 1980a. Method for the determination of 2,3,7,8-tetrachlorodibenzo-p-dioxin at ppt levels in vegetables by high resolution gas chromatography and low-resolution mass spectrometry. Chemosphere. 9(10): 623-628.

Cavallaro, A., G. Bandi, G. Invernizzi, L. Luciani, E. Mongini and A. Corni. 1980b. Sampling, occurrence and evaluation of PCDDs and PCDFs from incinerated solid urban waste. Chemosphere. 9(10): 611-621.

Cavallaro, A., G. Tebaldi and R. Gauldi. 1982. Analysis of transport and ground deposition of the TCDD emitted on 10 July 1976 from the ICMESA factory (Seveso, Italy). Atmos. Environ. 16: 731-740.

CEQ (Council on Environmental Quality). 1981. 12th Annual Report of the Council on Environmental Quality. Washington, DC. p. 129.

Chen, J-Y.T. 1973. Infrared studies of chlorinated dibenzo-p-dioxins and structurally related compound. J. Assoc. Off. Anal. Chem. 56: 962-975.

Cheney, B.V. 1982. Structural factors affecting the aryl hydrocarbon hydroxylase induction by dibenzo-p-dioxins and dibenzofurans. Int. J. Quantum Chem. XXI: 445-463.

Chess, E.K. and M.L. Gross. 1980. Determination of tetrachlorodibenzo-p-dioxins by mass spectrometric metastable decomposition monitoring. Anal. Chem. 52: 2057-2061.

Cheung, M.O., E.F. Gilbert and R.E. Petersen. 1981. Cardiovascular teratogenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the chick embryo. Toxicol. Appl. Pharmacol. 61: 197-204.

Chhabra, R.S., T.J. Michael, R.M. Philopot and J.R. Fouts. 1976. Relation between induction of aryl hydrocarbon hydroxylase and de novo synthesis of cytochrome P-448 (P1-450) in mice. Environ. Health Sci., Research Triangle Park, NC.

Chiappino, G., R. Gilioli and V. Foa. 1978. Report of Lombardy Regional Authority (unpublished). (Cited in Pocchiari et al., 1979)

Choudhary, G. 1983. Occupational exposure to polychlorinated dibenzo-p-dioxins and dibenzofurans: A perspective. In: Chlorinated Dioxins and Dibenzofurans in the Total Environment, G. Choudhary, L.H. Keith and C. Rappe, Ed. Butterworth Pubs., Boston, MA. p. 335-353.

Choudhry, G.G. and O. Hutzinger. 1984. Photochemical formation and degradation of polychlorinated dibenzofurans and dibenzo-p-dioxins. In: Residue Reviews. Residues of Pesticides and Other Contaminants in the Total Environment, F.A. Gunther and J.D. Gunther, Ed. Springer-Verlag, New York. Vol. 84, p. 113-161.

Clark, D.A., J. Gauldie, M.R. Szewczuk and G. Sweeney. 1981. Enhanced suppressor cell activity as a mechanism of immunosuppression by 2,3,7,8-tetrachlorodibenzo-p-dioxin (41275). Proc. Soc. Exp. Biol. Med. 168(2): 290-299.

Clark, D.A., G. Sweeney, S. Safe, E. Hancock, D.G. Kilburn and J. Gauldie. 1983. Cellular and genetic basis for suppression of cytotoxic T cell generation by haloaromatic hydrocarbons. Immunopharmacology. 6: 143-153.

Clement Associates. 1979. Exposure, toxicity and risk assessment of 2,4,5-T/TCDD. Prepared for U.S. EPA under contract no. 68-01-5095.

Clement, R.E. and F.W. Karasek. 1982. Distribution of organic compounds adsorbed on size-fractionated municipal incinerator fly ash particles. J. Chromatogr. 234: 395-405.

Cochrane, W.P., J. Singh, W. Miles and B. Wakeford. 1981. Determination of chlorinated dibenzo-p-dioxin contaminants in 2,4-D products by gas chromatography-mass spectrometric techniques. J. Chromatogr. 217: 289-299.

Cockerham, L.G., A.L. Young and C.E. Thalken. 1980. Histopathological and ultrastructural studies of liver tissue from TCDD-exposed beach mice (Peromyscus polionotus). J. AD-A083 323/6 PC A04/MF A01. 61 p.

Cocucci, S., F. Di Gerolamo, A. Verderio, et al. 1979. Absorption and translocation of tetrachlorodibenzo-p-dioxin by plants from polluted soil. Experientia. 35(4): 482-484.

Cohen, G.M., W.M. Bracken, R.P. Iyer, D.L. Berry, J.K. Selkirk and T.J. Slaga. 1979. Anticarcinogenic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on benzo(a)pyrene and 7,12-dimethylbenz(a)anthracene tumor initiation and its relationship to DNA binding. Cancer Res. 39(10): 4027-4033.

Collins, T.F.S., C.H. Williams and G.C. Gray. 1971. Teratogenic studies with 2,4,5-T and 2,4-D in the hamster. Bull. Environ. Contam. Toxicol. 6(6): 559-567.

Conway, C.C. and F. Matsumura. 1975. Alteration of cellular utilization of thymidine by TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin). Bull. Environ. Contam. Toxicol. 13(1): 52-56.

Cook R.R. 1981a. Dioxin, chloracne and soft-tissue sarcoma. Lancet. 1: 618-619.

Cook, R.R. 1981b. Mortality experience of employees to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Reply to comments. J. Occup. Med. 23(1): 8.

Cook, R.R., J.C. Townsend, M.G. Ott and L.G. Silverstein. 1980. Mortality experience of employees exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin. J. Occup. Med. 22(8): 530-532.

Cordle, F. 1981. The use of epidemiology in the regulation of dioxins in the food supply. Regulatory Toxicol. Pharmacol. 1: 379-387.

Cordle, F. 1983. Use of epidemiology in the regulation of dioxins in the food supply. In: Accidental Exposure to Dioxins: Human Health Aspects, F. Coulston and F. Pocchiari, Ed. Academic Press, NY. p. 245-256.

Coulston, F. and E.J. Olajos. 1980. Panel Report: Panel to Discuss the Epidemiology of 2,4,5-T. New York City, July 10-11, 1979. Ecotoxicol. Environ. Safety. 4: 96-102.

Courtney, K.D. 1976. Mouse teratology studies with chlorodibenzo-p-dioxins. Bull. Environ. Contam. Toxicol. 16(6): P674-681.

Courtney, K.D. 1977. Prenatal effects of herbicides evaluation by the prenatal development index. Arch. Environ. Contam. Toxicol. 6(1): 33.

Courtney, K.D. and J.A. Moore. 1971. Teratology studies with 2,4,5-T and 2,3,7,8-TCDD. Toxicol. Appl. Pharmacol. 20: 396-403.

Courtney, K.D., D.W. Gaylor, M.D. Hogan and H.L. Falk. 1970a. Teratogenic evaluation of pesticides: Large-scale screening study. Teratology. 3: 199.

Courtney, K.D., D.W. Gaylor, M.D. Hogan, M.L. Falk, R.R. Bates and I. Mitchell. 1970b. Teratogenic evaluation of 2,4,5-T. Science. 168: 864-866.

Courtney, K.D., J.P. Putnam and J.E. Andrews. 1978. Metabolic studies with TCDD (dioxin) treated rats. Arch. Environ. Contam. Toxicol. 7(4): 385-396.

Crampton, M.A. and L.J. Rogers. 1983. Low doses of 2,4,5-trichlorophenoxyacetic acid are behaviorally teratogenic to rats. Experientia. 39: 891-892.

Crosby, D.G. and A.S. Wong. 1976. Photochemical generation of chlorinated dioxins. Chemosphere. 5: 327-332.

Crosby, D.G. and A.S. Wong. 1977. Environmental degradation of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Science. 195: 1337-1338.

Crosby, D.G., A.S. Wong, J.R. Plimmer and E.A. Woolson. 1971. Photodecomposition of chlorinated dibenzo-p-dioxins. Science. 173(3998): 748-749.

Crow, K. 1978a. Chloracne: The chemical disease. New Sci. 78(1098): 78-80.

Crow, K.D. 1978b. Chloracne -- An up to date assessment. J. Ann Occup. Hyg. 21(3): 297-298.

Crow, K.D. 1981. Chloracne and its potential clinical implications. Clin. Exp. Dermatol. 6/3: 243-257.

Crummett, W.B. 1980. Dioxin detection. Nature. 283: 330.

Crummett, W.B. 1983. Status of analytical systems for the determination of PCDDs and PCDFs. Chemosphere. 12: 429-446.

Crummett, W.B. and R.H. Stehl. 1973. Determination of chlorinated dibenzo-p-dioxins and dibenzofurans in various materials. Environ. Health Perspect. 5: 15-25.

Crummett, W.B., R.R. Bumb, L.L. Lamparski, N.H. Mahle, T.J. Nestrick and L.W. Whiting. 1981. Environmental chlorinated dioxins from combustion: The trace chemistries of fire hypothesis. In: Impact of Chlorinated Dioxins and Related Compounds in the Environment, O. Hutzinger et al., Ed. Pergamon Press, Oxford. p. 253-263.

Crump, K.S. and W.W. Watson. 1979. GLOBAL 79: A Fortran program to extrapolate dichotomous animal carcinogenicity data to low dose. NIEHS, Contract No. I-ES-2123.

Crump, K.S., H.A. Givess and L.L. Deal. 1977. Confidence intervals and test of hypothesis concerning dose-response relations inferred from animal carcinogenicity data. Biometrics. 33: 437-451.

Cunningham, H.M. and D.T. Williams. 1972. Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin on growth rate and the synthesis of lipids and proteins in rats. Bull. Environ. Contam. Toxicol. 7(1): 45-51.

Cupitt, L.T. 1980. Fate of Toxic and Hazardous Materials in the Air Environment. EPA EPA 600/3-80-084, Environmental Sciences Research Laboratory, Research Triangle Park, NC.

Cutie, S.S. 1981. Recovery efficiency of 2,3,7,8-tetrachlorodibenzo-p-dioxin from active carbon and other particulates. Anal. Chim. Acta. 123: 25-31.

Czeizel, E. and J. Kiraly. 1976. Chromosome examinations in workers producing Klorinol and Buminol. In: The Development of a Pesticide as a Complex Scientific Task, L. Banki, Ed. Medicina: Budapest. p. 239-256. (Cited in NRCC, 1981a)

Czuczwa, J.M. and R.A. Hites. 1984. Environmental fate of combustion-generated polychlorinated dioxins and furans. Environ. Sci. Technol. 18(6): 444-450.

Czuczwa, J.M., B.D. McVeety and R.A. Hites. 1984. Polychlorinated dibenzo-p-dioxins and dibenzofurans in sediments from Siskiwit Lake, Isle Royale. Science. 226: 568-569.

Czuczwa, J.M., B.D. McVeety and R.A. Hites. 1985. Polychlorinated dibenzo-dioxins and dibenzofurans in sediments from Siskiwit Lake, Isle Royale. Chemosphere. 14(6/7): 623-626.

D'Argy, R., E. Hassoun and L. Dencker. 1984. Teratogenicity of TCDD and the congener 3,3'-4,4'-tetrachloroazobenzene in sensitive and non-sensitive mouse strains after reciprocal blastocyst transfer. Toxicol. Lett. 21: 197-202.

Dees, J.H., B.S. Masters, U. Muller-Eberhard and E.F. Johnson. 1982. Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin and phenobarbital on the occurrence and distribution of four cytochrome P-450 isozymes in rabbit kidney, lung, and liver. Cancer Res. 42(4): 1423-1432.

Deitrich, R.A., P. Bludeau, T. Stock and M. Roper. 1977. Induction of different rat liver supernatant aldehyde dehydrogenases by phenobarbital and tetrachlorodibenzo-p-dioxin. J. Biol. Chem. 252(17): 6169-6176.

Delgado, S. 1983. Division of Regulatory Guidance, Case and Advisory Branch, U.S. FDA. Personal communication.

Dencker, L. and R.M. Pratt. 1981. Association between the presence of the Ah-receptor in embryonic murine tissues and sensitivity to TCDD-induced cleft palate. Teratol. Carinog. Mutagen. 1: 399-406.

Department of Health, New Zealand. 1980. Report to the Minister of Health of an investigation into allegations of an association between human congenital defects and 2,4,5-T spraying in and around Te Kuiti. New Zealand Med. J. p. 314-315.

De Verneuil, H., S. Sassa and A. Kappas. 1983. Effects of polychlorinated biphenyl compounds, 2,3,7,8-tetrachlorodibenzo-p-dioxin, phenobarbital and iron on hepatic uroporphyrinogen decarboxylase. Biochem. J. 214: 145-151.

Dickens, M., M.D. Seefeld and R.E. Peterson. 1981. Enhanced liver DNA synthesis in partially hepateotomized rats pretreated with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol. Appl. Pharmacol. 58(3): 389-398.

DiDomenico, A., V. Silano, G. Viviano and G. Zapponi. 1980a. Accidental release of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) at Seveso, Italy. I. Sensitivity and specificity of analytical procedures adopted for TCDD assay. Ecotoxicol. Environ. Safety. 4(3): 283-297.

DiDomenico, A., V. Silano, G. Viviano and G. Zapponi. 1980b. Accidental release of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) as Seveso, Italy. VI. TCDD levels in atmospheric particles. Ecotoxicol. Environ. Safety. 4(3): 346-356.

DiDomenico, A., V. Silano, G. Viviano and G. Zapponi. 1980c. Accidental release of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) at Seveso, Italy. II. TCDD distribution in the soil surface layer. Ecotoxicol. Environ. Safety. 4(3): 298-320.

DiDomenico, A., V. Silano, G. Viviano and G. Zapponi. 1980d. Accidental release of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in Seveso, Italy. V. Environmental persistence of TCDD in soil. Ecotoxicol. Environ. Safety. 4(3): 339-345.

DiDomenico, A., V. Silano, G. Viviano and G. Zapponi. 1980e. Accidental release of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) at Seveso, Italy. IV. Vertical Distribution of TCDD in soil. Ecotoxicol. Environ. Safety. 4(3): 327-338.

DiDomenico, A., G. Viviano and G. Zapponi. 1982. Environmental persistence of 2,3,7,8-TCDD at Seveso. In: Chlorinated Dioxins and Related Compounds: Impact on the Environment, O. Hutzinger et al., Ed. Pergamon Press, NY. p. 105-114.

Dietrich, R.A., R. Bludeau, M. Roger and J. Schmuck. 1978. Induction of aldehyde dehydrogenases. Biochem. Pharmacol. 27: 2343-2347.

DiGiovanni, J., A. Viaje, D.L. Berry, T.J. Slaga and M.R. Juchau. 1977. Tumor-initiating ability of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and arochlor 1254 in the two-stage system of mouse skin carcinogenesis. Bull. Environ. Contam. Toxicol. 18(5): 552-557.

DiGiovanni, J., D.L. Berry, M.R. Juchau and T.J. Slaga. 1979a. 2,3,7,8-Tetrachlorodibenzo-p-dioxin: Potent anticarcinogenic activity in CD-1 mice. Biochem. Biophys. Res. Commun. 86(3): 577-584.

DiGiovanni, J., D.L. Berry, T.J. Slaga, A.H. Jones and M.R. Juchau. 1979b. Effects of pretreatment with 2,3,7,8-tetrachlorodibenzo-p-dioxin on the capacity of hepatic and extrahepatic mouse tissues to convert procarcinogens to mutagens for Salmonella typhimurium auxotrophs. Toxicol. Appl. Pharmacol. 50(2): 229-239.

DiGiovanni, J., D.L. Berry, G.L. Gleason, G.S. Kishore and T.J. Slaga. 1980. Time-dependent inhibition by 2,3,7,8-tetrachlorodibenzo-p-dioxin of skin tumorigenesis with polycyclic hydrocarbons. Cancer Res. 40(5): 1580-1587.

DiLernia, R., C. Crimaudo and G. Pacchetti. 1982. The study of X-rays and TCDD effects on satellite associations may suggest a simple model for application in environmental mutagenesis. Hum. Genet. 61(1): 42-47.

Dobbs, A.J. and C. Grant. 1979. Photolysis of highly chlorinated dibenzo-p-dioxins by sunlight. Nature. 278: 163-165.

Dougherty, W.J., M. Herbst and F. Coulston. 1975. The non-teratogenicity of 2,4,5-trichlorophenoxyacetic acid in the rhesus monkey, Macaca mulatta. Bull. Environ. Contam. Toxicol. 13: 477-482.

Dow Chemical Co. 1978. The trace chemistries of fire -- A source of and routes for the entry of chlorinated dioxins into the environment. The Chlorinated Dioxin Task Force, the Michigan Division, Dow Chemical, U.S.A.

Duran-Reynolds, M., F. Lillie, H. Bartsch and K.J. Blank. 1978. The genetic basis of susceptibility to leukemia induction in mice by 3-methylcholanthrene applied subcutaneously. J. Exp. Med. 147: 459-469.

Edling, C. and S. Granstam. 1979. XXXVI. National Conference of the Medical Society sponsored by the Swedish Medical Society at Stockholmsmasan, Alvifi, Stockholm, December 5-8, 1979. Summaries transaction of the Swedish Medical Society. 88(3): 73-74. (translation)

Eiceman, G.A., R.E. Clement and F.W. Karasek. 1979. Analysis of fly ash from municipal incinerators for trace organic compounds. Anal. Chem. 51(14): 2343-2350.

Eiceman, G.A., A.C. Viau and F.W. Karasek. 1980. Ultrasonic extraction of polychlorinated dibenzo-p-dioxins and other organic compounds from fly ash from municipal incinerators. Anal. Chem. 52: 1492-1496.

Eiceman, G.A., R.E. Clement and F.W. Karasek. 1981. Variations in concentrations of organic compounds including polychlorinated dibenzo-p-dioxins and polynuclear aromatic hydrocarbons in fly ash from a municipal incinerator. Anal. Chem. 53(7): 955-959.

Eisen, H.J. 1984. The nuclear TCDD:Ah receptor complex and the induction of cytochrome $P_1$-450 on RNA. In: Biological Mechanisms of Dioxin Action. Banbury Report 18. A. Poland and R.D. Kimbrough, Ed. Cold Spring Harbor Laboratory. p. 201-211.

Elovaara, E., H. Savolainen, M.G. Parkki, A. Aitio and H. Vainio. 1977. Neurochemical effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin in Wistar and Gunn rats. Research Commun. Chem. Pathol. Pharmacol. 18: 487-494.

Emerson, J.L., D.J. Thompson, C.G. Gerbig and V.B. Robinson. 1970. Teratogenic study of 2,4,5-trichlorophenoxyacetic acid in the rat. Toxicol. Appl. Pharmacol. 17: 317.

Emerson, J.L., D.J. Thompson, R.J. Strebing, C.G. Gerbig and B. Robinson. 1971. Teratogenic studies of 2,4,5-T in the rat and rabbit. Food Cosmet. Toxicol. 9: 395.

Environmental Canada. 1984. Chlorophenols and their impurities in the Canadian environment. 1983 Supplement. Economic and Technical Review Report. EPS3-EP-84-3. Environmental Protection Programs. Directorate, Canada.

Erickson, J.D., J. Mulinare, P.W. McClain, et al. 1984. Vietnam veterans' risks for fathering babies with birth defects. J. Am. Med. Assoc. 252(7): 903-912.

Eriksson, M., L. Hardell, N.O. Berg, T. Moller and O. Axelson. 1979. Case-control study on malignant mesenchymal tumors of the soft-tissue and exposure to chemical substances. Lakartidningen. 76: 3872-3875. (translation)

Eriksson, M., L. Hardell, N. O'Berg, T. Moller and O. Axelson. 1981. Soft-tissue sarcomas and exposure to chemical substances: A case-referent study. Br. J. Ind. Med. 38: 27-33.

Facchetti, S., A. Fornari and M. Montagna. 1980. Distribution of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the tissues of a person exposed to the toxic cloud at Seveso (Italy). Adv. Mass Spectrom. 8B: 1405-1414.

Faith, R.E. and M.I. Luster. 1979. Investigations of the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on parameters of various immune functions. Ann. NY Acad. Sci. 320: 564-571.

Fanelli, R., C. Chiabrando, M. Salmona, S. Garattini and P.G. Caldera. 1978. Degradation of 2,3,7,8-tetrachlorodibenzo-p-dioxin in organic solvents by gamma ray irradiation. Experientia. 34: 1126-1127.

Fanelli, R., M.P. Bertoni, M. Bonfanti, et al. 1980a. Routine analysis of 2,3,7,8-tetrachlorodibenzo-p-dioxin in biological samples from the contaminated area of Seveso, Italy. Bull. Environ. Contam. Toxicol. 24(6): 818-823.

Fanelli, R., M.P. Bertoni, M.G. Castelli, et al. 1980b. 2,3,7,8-Tetrachlorodibenzo-p-dioxin toxic effects and tissue levels in animals from the contaminated area of Seveso, Italy. Arch. Environ. Contam. Toxicol. 9(5): 569-577.

Fanelli, R., M.G. Castelli, G.P. Martelli, A. Noseda and S. Garattini. 1980c. Presence of 2,3,7,8-tetrachlorodibenzo-p-dioxin in wildlife living near Seveso, Italy: A preliminary study. Bull. Environ. Contam. Toxicol. 24(3): 460-462.

Fanelli, R., C. Chiabrando and A. Bonaccorsi. 1982. TCDD contamination in the Seveso incident. Drug Metab. Rev. 13: 407-422.

FDA (Food and Drug Administration). 1981. FDA advises Great Lakes states to monitor dioxin-contaminated fish. FDA talk paper dated August 28. In: Food Drug Cosmetic Law Reports, Paragraph 41, 321. Commerce Clearing House, Inc. September 8.

FDA (Food and Drug Administration). 1983. Statement by S.A. Miller, Director, Bureau of Foods, FDA, before The Subcommittee on Natural Resources, Agriculture Research and Environment, U.S. House of Representatives, June 30.

Federal Register. 1971. EPA Method 5. Determination of Particulate Emissions from Stationary Sources. 36: 24876-24895. December 23.

Federal Register. 1980a. Storage and Disposal of Waste Material; Prohibition of Disposal of Tetrachlorodibenzo-p-Dioxin. 45(98): 32676.

Federal Register. 1980b. Water Quality Criteria Documents; Availability. 45(231): 79318-79379.

Field, B. and C. Kerr. 1979. Herbicide use and incidence of neural-tube defects. Lancet. 1(8130): 1341-1342.

Fingerhut, M.A., W.E. Halperin, P.A. Honchar, A.B. Smith, D.H. Groth and W.O. Russell. 1984. An evaluation of reports of dioxin exposure and soft tissue sarcoma pathology in U.S. chemical workers. In: Biological Mechanisms of Dioxin Action. Banbury Report 18. A. Poland and R.D. Kimbrough, Ed. Cold Spring Harbor Laboratory. p. 461-470.

Finney, D.J. 1971. Probit Analysis. Cambridge University Press, London. 333 p.

Firestone, D. 1973. Etiology of chick edema disease. Environ. Health Perspect. 5: 59-66.

Firestone, D. 1977a. Chemistry and analysis of pentachlorophenol and its contaminants. FDA By-lines. 2: 57-89.

Firestone, D. 1977b. Determination of polychlorodibenzo-p-dioxins and polychlorodibenzofurans in commercial gelatins by gas-liquid chromatography. J. Agric. Food Chem. 25: 1274-1280.

Firestone, D., J. Ress, N.L. Brown, R.P. Barron and J.N. Damico. 1972. Determination of polychlorodibenzo-p-dioxins and related compounds in commercial chlorophenols. J. Assoc. Off. Anal. Chem. 55(1): 85-92.

Firestone, D., M. Clower, Jr., A.P. Borsetti, R.H. Teske and P.E. Long. 1979. Polychlorodibenzo-p-dioxin and pentachlorophenol residues in milk and blood of cows fed technical pentachlorophenol. J. Agric. Food Chem. 27(6): 1171-1177.

Fowler, B.A., G.W. Lucier, H.W. Brown and O.S. McDaniel. 1973. Ultrastructural changes in rat liver cells following a single oral dose of TCDD. Environ. Health Perspect. 5: 141-148.

Fries, G.F. and G.S. Marrow. 1975. Retention and excretion of 2,3,7,8-tetrachlorodibenzo-p-dioxin by rats. J. Agric. Food Chem. 23(2): 265-269.

Garattini, S. 1982. TCDD toxicology with particular reference to Seveso: Introductory remarks. Drug Metab. Rev. 13(3): 345-353.

Gasiewicz, T.A. and R.A. Neal. 1979. 2,3,7,8-Tetrachlorodibenzo-p-dioxin tissue distribution, excretion, and effects on clinical chemical parameters in guinea pigs. Toxicol. Appl. Pharmacol. 51(2): 329-340.

Gasiewicz, T.A. and R.A. Neal. 1982. The examination and quantitation of tissue cytosolic receptors for 2,3,7,8-tetrachlorodibenzo-p-dioxin using hydroxyapatite. Anal. Biochem. 124: 1-11.

Gasiewicz, T.A., M.A. Holscher and R.A. Neal. 1980. The effect of total parenteral nutrition on the toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the rat. Toxicol. Appl. Pharmacol. 54: 469-488.

Gasiewicz, T.A., J.R. Olson, L.E. Geiger and R.A. Neal. 1983a. Absorption, distribution and metabolism of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in experimental animals. In: Human and Environmental Risks of Chlorinated Dioxins and Related Compounds, R.E. Tucker, A.L. Young and A.P. Gray, Ed. Plenum Press, NY. p. 495-525.

Gasiewicz, T.A., L.E. Geiger, G. Rucci and R.A. Neal. 1983b. Distribution, excretion, and metabolism of 2,3,7,8-tetrachlorodibenzo-p-dioxins in C57Bl/6J, DBA/2J and $B6D2F_1$/J mice. Drug Metab. Dispos. (In press)

Gebefuegi, I., R. Baumann and F. Korte. 1977. Photochemical degradation of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) under simulated environmental conditions. Naturwissenschaften. 64(9): 486-487.

Geiger, L.E. and R.A. Neal. 1981. Mutagenicity testing of 2,3,7,8-tetrachlorodibenzo-p-dioxin in histidine auxotrophs of Salmonella typhimurium. Toxicol. Appl. Pharmacol. 59(1): 125-129.

Ghezzi, I., P. Cannatelli, G. Assennato, et al. 1982. Potential 2,3,7,8-tetrachlorodibenzo-p-dioxin exposure of Seveso decontamination workers: A controlled prospective study. Scand. J. Work Environ. Health. 8(Suppl. 1): 176-179.

Gianotti, F. 1977. Chloracne due to tetrachloro-2,3,7,8-dibenzo-p-dioxin in children. Ann. Dermatol. Venereol. 104(12): 825-829.

Giavini, E., M. Prati and C. Vismara. 1982a. Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin administered to pregnant rats during the preimplantation period. Environ. Res. 29(1): 185-189.

Giavini, E., M. Prati and C. Vismara. 1982b. Rabbit teratology study with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Environ. Res. 27(1): 74-78.

Giavini, E., M. Prati and C. Vismara. 1983. Embryotoxic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin administered to female rats before mating. Environ. Res. 31: 105-110.

Gilbert, P., G. Saint-Ruf, F. Poncelet and M. Mercier. 1980. Genetic effects of chlorinated anilines and azobenzenes on Salmonella typhimurium. Arch. Environ. Contam. Toxicol. 9(5): 533-541.

Gizzi, F., R. Reginato, E. Benfenati and R. Fanelli. 1982. Polychlorinated dibenzo-p-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) in emissions from an urban incinerator. I. Average and peak values. Chemosphere. 11: 577-583.

Glaser, J.A., D.L. Foerst, G.D. McKee, S.A. Quave and W.L. Budde. 1981. Trace analyses for wastewaters. Environ. Sci. Technol. 15(12): 1426.

Goldstein, J.A., P. Hickman, H. Bergman, J.D. McKinney and M.P. Walker. 1977. Separation of pure polychlorinated biphenyl isomers into two types of inducers on the basis of induction of cytochrome P-450 or P-448. Chem.-Biol. Interact. 17: 69-87.

Goldstein, J.A., M. Friesen, T.M. Scotti, P. Hickman, J.R. Hass and H. Bergman. 1978. Assessment of the contribution of chlorinated dibenzo-p-dioxins and dibenzofurans to hexachlorobenzene-induced toxicity, porphyria, changes in mixed function oxygenases, and histopathological changes. Toxicol. Appl. Pharmacol. 46(3): 633-649.

Goldstein, J.A., P. Linko, J.N. Huckins and D.L. Stalling. 1982a. Structure-activity relationships of chlorinated benzenes as inducers of different forms of cytochrome P-450 in rat liver. Chem. Biol. Interact. 41(2): 131-139.

Goldstein, J.A., P. Linko and H. Bergman. 1982b. Induction of porphyria in the rat by chronic versus acute exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Biochem. Pharmacol. 31(8): 1607-1613.

Gonzalez, F.J., R.H. Tukey and D.W. Nebert. 1984. Structural gene products of the Ah locus. Transcriptional regulation of cytochrome $P_1$-450 and $P_3$-450 mRNA levels by 3-methylcholanthrene. Mol. Pharmacol. 26: 117-121.

Gorski, T. 1981. Presence of polychlorinated dibenzo-p-dioxins in latex nipples. Bull. Environ. Contam. Toxicol. 27: 68-71.

Gray, A.P., S.P. Cepa and J.S. Cantrell. 1975. Intervention of the Smiles rearrangement in synthesis of dibenzo-p-dioxins: 1,2,3,6,7,8- and 1,2,3,7,8,9-hexachlorodibenzo-p-dioxin (HCDD). Tetrahedron Lett. 33: 2873-2876.

Gray, A.P., S.P. Cepa, I.J. Solomon and O. Aniline. 1976. Synthesis of specific polychlorinated dibenzo-p-dioxins. J. Org. Chem. 41: 2435-2437.

Green, S. and F.S. Moreland. 1975. Cytogenetic evaluation of several dioxins in the rat. Toxicol. Appl. Pharmacol. 33: 161.

Green, S., F. Moreland and C. Sheu. 1977. Cytogenic effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin on rat bone marrow cells. U.S. FDA, Washington, DC. FDA By-Lines. 6: 292.

Greenlee, W.F. and A. Poland. 1978. An improved assay of 7-ethoxycoumarin O-deethylase activity: Induction of hepatic enzyme activity in C57Bl/6J and DBA/2J mice by phenobarbital, 3-methylcholanthrene and 2,3,7,8-tetrachlorodibenzo-p-dioxin. J. Pharmacol. Exp. Ther. 205(3): 596-605.

Greenlee, W.F. and A. Poland. 1979. Nuclear uptake of 2,3,7,8-tetrachlorodibenzo-p-dioxin in C57Bl/6J and DBA/2J mice. Role of the hepatic cytosol receptor protein. J. Biol. Chem. 254(19): 9814-9821.

Greig, J.B. 1972. Effect of 2,3,7,8-tetrachlorodibenzo-1,4-dioxin on drug metabolism in the rat. Biochem. Pharmacol. 21(23): 3196-3198.

Greig, J.B., G. Jones, W.H. Butler and J.M. Barnes. 1973. Toxic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Food Cosmet. Toxicol. 11: 585-595.

Greig, J.B., D.M. Taylor and J.D. Jones. 1974. Effects of 2,3,7,8-tetrachloro-dibenzo-p-dioxin on stimulated DNA synthesis in the liver and kidney of the rat. Chem. Biol. Interact. 8(1): 31-39.

Gross, M.L., T. Sun, P.A. Lyon, et al. 1981. Method validation for the determination of tetrachlorodibenzodioxin at the low parts-per-trillion level. Anal. Chem. 53(12): 1902-1906.

Gross, M.L., J.O. Lay, Jr., P.A. Lyon, et al. 1984. 2,3,7,8-Tetrachlorodibenzo-p-dioxin levels in adipose tissue of Vietnam veterans. Environ. Res. 33: 261-268.

Guenthner, T.M. and D.W. Nebert. 1977. Cytosolic receptor for aryl hydrocarbon hydroxylase induction by polycyclic aromatic compounds. J. Biol. Chem. 252: 8981-8989.

Guenthner, T.M., J.M. Fysh and D.W. Nebert. 1979a. 2,3,7,8-Tetrachlorodibenzo-p-dioxin: Covalent binding of reactive metabolic intermediates principally to protein *in vitro*. Pharmacology. 19: 12-22.

Guenthner, T.M., D.W. Nebert and R.H. Menard. 1979b. Microsomal aryl hydrocarbon hydroxylase in rat adrenal: Regulation by ACTH but not by polycyclic hydrocarbons. Mol. Pharmacol. 15(3): 719-728.

Gupta, B.N., J.G. Vos, J.A. Moore, J.G. Zinkl and B.C. Bullock. 1973. Pathologic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin in laboratory animals. Environ. Health Perspect. 5: 125-140.

Gustafsson, J.A. and M. Ingelman-Sundberg. 1979. Changes in steroid hormone metabolism in rat liver microsomes following administration of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Biochem. Pharmacol. 28(4): 497-499.

Hailey, C.L., J.S. Stanley, A.M. Magin, R.V. Northcutt and D.P. Redford. 1983. Emissions of Organic Pollutants from Coal-Fired Utility Boiler Plants. Presented at the ACS Annual Meeting, Seattle, WA. March 20-25.

Hajdu, S.I. 1983. Classification of soft tissue tumors and tumor-like lesions. Presented at Symposium on Public Health Risks of the Dioxins, Rockefeller Univ., October 19-20.

Häkansson, H. and U.G. Ahlborg. 1985. The effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on the uptake, distribution and excretion of a single oral dose of [11,12-$^3$H]retinyl acetate and on the vitamin A status in the rat. J. Nutr. 115: 759-771.

Hallett, D. 1984. Environment Canada, Toronto, Ontario. Private communication with Dr. S.H. Safe, Texasd A&M Univ., College Station, TX.

Hanify, J.A., P. Metcalf, C.L. Nobbs and R.J. Worsley. 1981. Aerial spraying of 2,4,5-T and human birth malformations: An epidemiological investigation. Science. 212: 349-351.

Hardell, L. 1977. Malignant mesenchymal tumors and exposure to phenoxyacids: A clinical observation. Lakartidningen. 74: 2753-2754. (translation)

Hardell, L. 1979. Malignant lymphoma of histiocytic type and exposure to phenoxyacetic acids or chlorophenols. Lancet. 1: 55-56.

Hardell, L. 1981. Relation of soft-tissue sarcoma, malignant lymphoma and colon cancer to phenoxy acids, chlorophenols and other agents. Scand. J. Work Environ. Health. 7: 119-130.

Hardell, L. 1983. Letter to D. Bayliss, Carcinogen Assessment Group, U.S. EPA, Washington, DC, August 5.

Hardell, L. and M. Eriksson. 1981. Soft-tissue sarcomas, phenoxy herbicides, and chlorinated phenols. Lancet. 2(8240): 8/1/81.

Hardell, L. and A. Sandstrom. 1979. Case-control study: Soft-tissue sarcomas and exposure to phenoxyacetic acids or chlorophenols. Br. J. Cancer. 39: 711-717.

Hardell, L., M. Eriksson and P. Lenner. 1980. Malignant lymphoma and exposure to chemical substances, especially organic solvents, chlorophenols, and phenoxy acids. Lakartidningen. 77: 208-210. (translation)

Hardell, L., M. Eriksson, P. Lenner and E. Lundgren. 1981. Malignant lymphoma and exposure to chemicals, especially organic solvents, chlorophenols and phenoxy acids: A case-control study. Br. J. Cancer. 43: 169-176.

Harless, R. 1981. Internal memo to M. Dellarco. Office of Toxic Substances, U.S. EPA, Washington, DC.

Harless, R.L. and R.G. Lewis. 1980a. Quantitative capillary column gas chromatography-mass spectrometry methods of analysis for toxic organic compounds. Sym.: Practical Solutions to Quantitative Capillary Column Gas Chromatography. Cong.: Anal. Chem. Appl. Spectro., Atlantic City, NJ. March, 1980.

Harless, R.L. and R.G. Lewis. 1980b. Quantitative determination of 2,3,7,8-tetrachlorodibenzo-p-dioxin residues by gas chromatography/mass spectrometry. Presented at Workshop on Impact of Chlorinated Dioxins and Related Compounds on the Environment. Instituto Superiore di Sanita, Rome, Italy. October 22-24.

Harless, R.L. and R.G. Lewis. 1982. Quantitative determination of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) isomers. In: Chlorinated Dioxins and Related Compounds. Impact on the Environment, O. Hutzinger, R.W. Frei, E. Merian and F. Pocchiari, Ed. Pergamon Press, NY. p. 25-35.

Harless, R.L. and E.O. Oswald. 1978. Gas chromatograpy/mass spectrometry interface for glass capillary columns. Paper presented at the 26th Annual Conference Mass Spectrometry. May 28-June 2, 1978.

Harless, R.L., E.O. Oswald, M.K. Wilkinson, et al. 1980. Sample preparation and gas chromatography-mass spectrometry determination of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Anal. Chem. 52(8): 1239-1245.

Harris, H.W., J.A. Moore, J.G. Vos and B.N. Gupta. 1973. General biological effects of TCDD in laboratory animals. Environ. Health. Perspect. 5: 101-109.

Harvan, D.J., J.R. Hass, J.L. Schroeder and B.J. Corbett. 1981. Detection of tetrachlorodibenzodioxins in air filter samples. Anal. Chem. 53(12): 1755-1759.

Hass, J.R. and M.D. Friesen. 1979. Qualitative and quantitative methods for dioxin analysis. Ann. NY Acad. Sci. 320: 28-42.

Hass, J.R., M.D. Friesen, D.J. Harvan and C.E. Parker. 1978. Determination of polychlorinated dibenzo-p-dioxins in biological samples by negative chemical ionization mass spectrometry. Anal. Chem. 50(11): 1474-1479.

Hassan, M.Q., S.J. Stohs and W.J. Murray. 1985. Effects of vitamins E and A on 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-induced lipid peroxidation and other biochemical changes in the rat. Arch. Environ. Contam. Toxicol. 14: 437-442.

Hassoun, E.M. and L. Dencker. 1982. TCDD embryotoxicity in the mouse may be enhanced by β-naphthflovone, another ligand of the Ah-receptor. Toxic. Lett. 12: 191-198.

Hatch, M.C. 1984. Reproductive effects of the dioxins. In: Public Health Risks of the Dioxins, W.W. Lawrence, Ed. William Kaufmann, Los Altos, CA. p. 255-271.

Hawkes, C.L. and L.A. Norris. 1977. Chronic oral toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) to rainbow trout. Trans. Am. Fish Soc. 106(6): 641-645.

Hay, A. 1979. Dispute over Dow Chemical's theory of dioxin traces. Nature. 281: 619-620.

Hay, A. 1982. Toxicology of dioxins. In: The Chemical Scythe: Lessons of 2,4,5-T and Dioxin. Plenum Press, NY and London. p. 41-47.

Heida, H. 1983. TCDD in bottom sediments and eel around a refuse dump near Amsterdam, Holland. Chemosphere. 12: 503-509.

Heath, R.G.E., R.L. Harless, M.L. Gross, P.A. Lyon, A.E. Dupuy, Jr. and D.D. McDaniel. 1985. Determination of 2,3,7,8-TCDD in human milk at the 0.1 to 10 ppt level: Method validation and survey results. (Submitted for publication)

Helder, T. 1980. Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on early life stages of the pike (Esox lucius L.). Sci. Total Environ. 14(3): 255-264.

Helder, T. 1981. Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on early life stages of rainbow trout (Salmo gairdneri, Richardson). Toxicol. 19(2): 101-112.

Helling, C.S., A.R. Isensee, E.A. Woolson, et al. 1973. Chlorodioxins in pesticides, soils and plants. J. Environ. Quality. 2(2): 171-178.

Henck, J.W., M.A. New, R.J. Kociba and K.S. Rao. 1981. 2,3,7,8-Tetrachlorodibenzo-p-dioxin: Acute oral toxicity in hamsters. Toxicol. Appl. Pharmacol. 59: 405-407.

Hildebrandt, P.K. 1983. Letter to E.E. McConnell, NTP, October 31, 1983, with attached tables.

Hiles, R.A. and R.D. Bruce. 1976. 2,3,7,8-Tetrachlorodibenzo-p-dioxin elimination in the rat: First order or zero order? Food Cosmet. Toxicol. 14(6): 599-600.

Hinsdill, R.D., D.L. Couch and R.S. Speirs. 1980. Immunosuppression in mice induced by dioxin (TCDD) in feed. J. Environ. Pathol. Toxicol. 4(2-3): 401-425.

Holmstedt, B. 1980. Prolegomena to Seveso Ecclesiastes I 18. Arch. Toxicol. 44(4): 211-230.

Honchar, P.A. and W.E. Halperin. 1981. 2,4,5-Trichlorophenol and soft-tissue sarcoma. Lancet. 1(8214): 268-269.

Hook, G.E.R., J.K. Haseman and G.W. Lucier. 1975a. Induction and suppression of hepatic and extrahepatic microsomal foreign-compound-metabolizing systems by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Chem.-Biol. Interact. 10(3): 199-214.

Hook, G.E.R., T.C. Orton, J.A. Moore and G.W. Lucier. 1975b. Tetrachlorodibenzo-p-dioxin-induced changes in the hydroxylation of biphenyl by rat liver microsomes. Biochem. Pharmacol. 24(3): 335-340.

Hook, J.B., K.M. McCormack and W.M. Kluwe. 1977. Renal effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin. In: Pentachlorophenol, K.R. Rao, Ed. Plenum Press, NY. p. 381-387.

Hope, W., D. Lischwe, R. Russell and S. Weiss. 1984. Porphyrin cutanea tarda and sarcoma in a worker exposed to 2,3,7,8-tetrachlorodibenzodioxin. Missouri J. Am. Med. Assoc. 251(12): 1534, 1537.

Houk, V.N. 1983. Testimony by Dr. Vernon W. Houk, Director, Center for Environmental Health, Center for Disease Control, Public Health Service, Department of Health and Human Services before the United States House of Representatives Science and Technology Committee, Subcommittee on Natural Resources, Agricultural Research and Environment. March 23.

Hryhorczuk, D.O., W.A. Withrow, C.S. Hesse and V.R. Beasley. 1981. Wire reclamation incinerator as a source of environmental contamination with tetrachlorodibenzo-p-dioxins and tetrachlorodibenzofurans. Arch. Environ. Health. 36(5): 228-234.

Huckins, J.N., D.L. Stalling and W.A. Smith. 1978. Foam-charcoal chromatography for analysis of polychlorinated dibenzodioxins in Herbicide Orange. Assoc. Off. Anal. Chem. 61(1): 32-38.

Hudson, L.G., R. Shaikh, W.A. Toscano and W.F. Greenlee. 1983. Induction of 7-ethoxycoumarin o-deethylase activity in cultured human epithelial cells by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD): Evidence for TCDD receptor. Biochem. Biophys. Res. Comm. 115: 611-617.

Hueper, W.C. and W.D. Conway. 1964. Chemical Carcinogenesis and Cancers. Springfield, Thomas.

Huetter, R. and M. Philippi. 1982. Studies on microbial metabolism of TCDD under laboratory conditions. Pergamon Ser. Environ. Sci. 5: 87-93.

Hummel, R.A. 1977. Clean-up techniques for the determination of parts per trillion residue levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). J. Agric. Food Chem. 25: 1049-1053.

Hummel, R.A. and L.A. Shadoff. 1980. Specificity of low resolution gas chromatography-low resolution mass spectrometry for the detection of tetrachlorodibenzo-p-dioxin in environmental samples. Anal. Chem. 52: 191-192.

Hussain, S., L. Ehrenberg, G. Lofroth and T. Gejvall. 1972. Mutagenic effects of TCDD on bacterial systems. Ambio. 1: 32-33.

Hutzinger, O., K. Olie, J.W. Lustenhouwer, A.B. Okey, S. Bandiera and S. Safe. 1981. Polychlorinated dibenzo-p-dioxins and dibenzofurans: A bioanalytical approach. Chemosphere. 10(1): 19-25.

Hwang, S.W. 1973. Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin on the biliary excretion of indocyanine green in rat. Environ. Health Perspect. 5: 227-231.

IARC (International Agency for Research on Cancer). 1977. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man. Some Fumigants, the Herbicides 2,4-D and 2,4,5-T, Chlorinated Dibenzodioxins and Miscellaneous Industrial Chemicals. Vol. 15, IARC, Lyon, France. p. 41-102.

ICD (International Classification of Diseases). 1975. 9th revision. WHO, Geneva.

ICRP (International Commission on Radiological Protection). 1959. Publication 2. Report of Committee on Permissible Dose for Internal Radiation. Pergamon Press, London.

Ideo, G., G. Bellati, A. Bellobuono, P. Mocarelli, A. Marocchi and P. Brambilla. 1982. Increased urinary D-glucaric acid excretion by children living in an area polluted with tetrachlorodibenzoparadioxin (TCDD). Clin. Chim. Acta. 120(3): 273-283.

Isensee, A.R. 1978. Bioaccumulation of 2,3,7,8-tetrachlorodibenzo-para-dioxin. Ecol. Bull. C. Ramel, Ed. 27: 255-262.

Isensee, A.R. and G.E. Jones. 1971. Absorption and translocation of root and foliage applied 2,4-dichlorophenol, 2,7-dichlorodibenzo-p-dioxin, and 2,3,7,8-tetrachlorodibenzo-p-dioxin. J. Agric. Food Chem. 19: 1210-1214.

Isensee, A.R. and G.E. Jones. 1975. Distribution of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in aquatic model ecosystem. Environ. Sci. Technol. 9: 668-672.

Ishidate, K., M. Tsuruoka and Y. Nakazawa. 1980. Induction of choline kinase by polycyclic aromatic hydrocarbon carcinogens in rat liver. Biochem. Biophys. Res. Commun. 96: 946-952.

Jackson, W.T. 1972. Regulation of mitosis. III. Cytological effects of 2,4,5-trichlorophenoxyacetic acid and of dioxin contaminants in 2,4,5-T formulations. J. Cell Sci. 10: 15-25.

Jensen, D.J. and R.A. Hummel. 1982. Secretion of TCDD in milk and cream following the feeding of TCDD to lactating dairy cows. Bull. Environ. Contam. Toxicol. 29: 440-446.

Jensen, D.J., R.A. Hummel, N.H. Mahle, C.W. Kocher and H.S. Higgins. 1981. Residue study on beef cattle consuming 2,3,7,8-tetrachlorodibenzo-p-dioxin. J. Agric. Food Chem. 29(2): 265-268.

Jensen, D.J., M.E. Getzendaner, R.A. Hummel and J. Turley. 1983. Residue studies for (2,4,5-trichlorophenoxy) acetic acid and 2,3,7,8-tetrachlorodibenzo-p-dioxin in grass and rice. J. Agric. Food Chem. 31: 118-122.

Jett, G. 1982. Memorandum to J.S. Bellin (Office of Solid Waste, U.S. EPA, Washington, DC). Re: Hazardous waste estimates for phenoxyacetic compounds and priority pollutant chlorophenols in the pesticide industry. June 1.

Johnson, E.F. and U. Muller-Eberhard. 1977a. Resolution of two forms of cytochrome P-450 from liver microsomes of rabbits treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin. J. Biol. Chem. 252(9): 2839-2845.

Johnson, E.F. and U. Muller-Eberhard. 1977b. Multiple forms of cytochrome P-450 from liver microsomes of rabbits treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (Meeting Abstract). Fed. Proc. 36(3): 833.

Johnson, E.F. and U. Muller-Eberhard. 1977c. Purification of the major cytochrome P-450 of liver microsomes from rabbits treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Biophys. Res. Commun. 76(3): 652-659.

Johnson, E.F. and U. Muller-Eberhard. 1977d. Multiple forms of cytochrome P-450 resolution and purification of rabbit liver aryl hydrocarbon hydroxylase. Biochem. Biophys. Res. Commun. 76(3): 644-651.

Johnson, E.F., G.E. Schwab and U. Muller-Eberhard. 1979. Multiple forms of cytochrome P-450: Catalytic differences exhibited by two homogeneous forms of rabbit cytochrome P-450. Mol. Pharmacol. 15(3): 708-718.

Johnson, F.E., M.A. Kugler and S.M. Brown. 1981. Soft-tissue sarcomas and chlorinated phenols. Lancet. 2(8236): 40.

Johnson, R.M. and C.A. Baumann. 1947. Storage and distribution of vitamin A in rats fed certain isomers of carotene. Arch. Biochem. 14(3): 361-367.

Jones, G. 1975. A histochemical study of the liver lesion induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin) in rats. J. Pathol. 116(2): 101-105.

Jones, G. and W.H. Butler. 1974. A morphological study of the liver lesion induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin in rats. J. Pathol. 112: 93-97.

Jones, G. and J.B. Greig. 1975. Pathological changes in the liver of mice given 2,3,7,8-tetrachlorodibenzo-p-dioxin. Experientia. 31(11): 1315-1317.

Jones, K.G. and G.D. Sweeney. 1977. Association between induction of aryl hydrocarbon hydroxylase and depression of uroporphyrinogen decarboxylase activity. Res. Commun. Chem. Pathol. Pharmacol. 17(4): 631-638.

Jones, K.G. and G.D. Sweeney. 1980. Dependence of the porphyrogenic effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin upon inheritance of aryl hydrocarbon hydroxylase responsiveness. Toxicol. Appl. Pharmacol. 53(1): 42-49.

Josephson, J. 1983. Chlorinated dioxins and furans in the environment. Environ. Sci. Technol. 17: 124A-128A.

Junk, G.A. and J.J. Richard. 1981. Dioxins not detected in effluents from coal/refuse combustion. Chemosphere. 10: 237-241.

Kapitulnik, J. and J.D. Ostrow. 1978. Stimulation of bilirubin catabolism in jaundiced Gunn rats by an inducer of microsmal mixed-function monooxygenases. Proc. Natl. Acad. Sci. 75(2): 682-685.

Karasek, F.W., R.E. Clement and A.C. Vian. 1982. Distribution of PCDDs and other toxic compounds generated on fly ash particulates in municipal incinerators. J. Chromatogr. 239: 173-180.

Karickhoff, S.W., D.S. Brown and T.A. Scott. 1979. Sorption of hydropholic pollutants on natural sediments. Water Res. 13: 241-248.

Kearney, P.C., E.A. Woolson and C.P. Ellington, Jr. 1972. Persistence and metabolism of chlorodioxins in soils. Environ. Sci. Technol. 6(12): 1017-1019.

Kenaga, E.E. 1980. Correlation of bioconcentration factors of chemicals in aquatic and terrestrial organisms with their physical and chemical properties. Environ. Sci. Technol. 14: 553-556.

Kenaga, E.E. and C.A.I. Goring. 1980. Relationship between water solubility, soil sorption, octanol-water partitioning, and concentration of chemicals in biota. In: J.G. Eaton et al., Ed., Aquatic Toxicology ASTM STP 707, American Society for Testing and Materials, Philadelphia, PA. p. 78-115.

Kende, A.S. and J.J. Wade. 1973. Synthesis of new stemic and electronic analogs of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Environ. Health Perspect. 5: 49-57.

Kende, A.S., J.J. Wade, D. Ridge and A. Poland. 1974. Synthesis and Fourier transform carbon-13 nuclear magnetic resonance spectroscopy of new toxic polyhalodibenzo-p-dioxins. J. Org. Chem. 39: 931-937.

Khera, K.S. and W.P. McKinley. 1972. Pre- and postnatal studies on 2,4,5-T and 2,4-D and their derivatives in rats. Toxicol. Appl. Pharmacol. 22: 14.

Khera, K.S. and J.A. Ruddick. 1973. Polychlorodibenzo-p-dioxins: Perinatal effects and the dominant lethal test in Wistar rats. In: Chlorodioxins -- Origins and Fate, E.H. Blair, Ed. Adv. Chem. Ser. No. 120. Am. Chem. Soc., Washington, DC. p. 70-84.

Khera, K.S., B.L. Huston and W.P. McKinley. 1971. Pre- and postnatal studies on 2,4,5-T, 2,4-D, and derivatives in Wistar rats. Toxicol. Appl. Pharmacol. 19: 369-370.

Kim, P., G.C. Yang, D. Firestone and A.E. Pohland. 1975. Photochemical Dechlorination of Chlorinated Dibenzo-p-dioxins. Presented at the 1st Chemical Congress of the North American Continent, Mexico City, November.

Kimble, B.J. and M.L. Gross. 1980. Tetrachlorodibenzo-p-dioxin quantitation in stack-collected coal fly ash. Science. 207: 59-61.

Kimbrough, R.D. 1974. The toxicity of polychlorinated polycyclic compounds and related chemicals. CRC Crit. Rev. Toxicol. 2: 445-489.

Kimbrough, R.D., C.D. Carter, J.A. Liddle and R.E. Cline. 1977. Epidemiology and pathology of a tetrachlorodibenzodioxin poisoning episode. Arch. Environ. Health. 32(2): 77-86.

Kimbrough, R.D., J. Buckley, L. Fishbein, et al. 1978. Animal toxicology. Environ. Health Perspect. 24: 173-184.

Kimbrough, R.D., H. Falk, P. Stehr and G. Fries. 1984. Health implications of 2,3,7,8-tetrachlorodibenzodioxin (TCDD) contamination of residential soil. J. Toxicol. Environ. Health. 14: 47-93.

Kimmig, J. and K.H. Schulz. 1957. Berufliche akna (sog. chlorakne) durch chloriette aromatische zyklische ather. Dermatologia. 115: 540-546. (Ger.)

Kimura, S., F.J. Gonzalez and D.W. Nebert. 1984. The murine Ah locus: Comparison of the complete cytochrome $P_1$-450 and $P_3$-450 cDNA nucleotide and amino acid sequences. J. Biol. Chem. 259(17): 10705-10713.

King, M.E. and A.R. Roesler. 1974. Subacute intubation study on rats with the compound 2,3,7,8-tetrachlorodioxin. U.S. EPA. NTIS PB-257 677. p. 27.

Kirsch, et al. 1975. Structural and functional studies of ligandin, a major renal organic anion-binding protein. J. Clin. Invest. 55: 1009.

Kitchin, K.T. and J.S. Woods. 1977. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) induced synthesis of cytochrome P-448 and aryl hydrocarbons hydroxylase (AHH) activity in female rat liver (Meeting Abstract). Pharmacol. 19(2): 232.

Kitchin, K.T. and J.S. Woods. 1978a. 2,3,7,8-Tetrachlorodibenzo-p-dioxin induction of aryl hydrocarbon hydroxylase (AHH) in hepatic microsomes from female rats. Toxicol. Appl. Pharmacol. 45(1): 297.

Kitchin, K.T. and J.S. Woods. 1978b. 2,3,7,8-Tetrachlorodibenzo-p-dioxin induction of aryl hydrocarbon hydroxylase in female rat liver, evidence for de novo synthesis of cytochrome P-448. Mol. Pharmacol. 14: 890-899.

Knutson, J.C. and A. Poland. 1980. 2,3,7,8-Tetrachlorodibenzo-p-dioxin: Failure to demonstrate toxicity in twenty-three cultured cell types. Toxicol. Appl. Pharmacol. 54(3): 377-383.

Knutson, J.C. and A. Poland. 1982. Response of murine epidermis to the 2,3,7,8-tetrachlorodibenzo-p-dioxin: Interaction of the Ah and hr loci. Cell. 30(1): 225-234.

Kocher, C.W., N.H. Mahle, R.A. Hummel, L.A. Shadoff and M.E. Getzendaner. 1978. A search for the presence of 2,3,7,8-tetrachlorodibenzo-p-dioxin in beef fat. Bull. Environ. Contam. Toxicol. 19: 229-236.

Kociba, R.J. and B.A. Schwetz. 1982. Toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Drug Metab. Rev. 13: 387-406.

Kociba, R.J., P.A. Keeler, C.N. Park and P.J. Gehring. 1976. 2,3,7,8-Tetrachlorodibenzo-p-dioxin results of a 13-week oral toxicity study in rats. Toxicol. Appl. Pharmacol. 35: 553-573.

Kociba, R.J., D.G. Keyes, J.E. Beyer, et al. 1977. Results of a two-year chronic toxicity and oncogenicity study of 2,3,7,8-tetrachlorodibenzo-p-dioxin in rats. Unpublished report submitted to U.S. EPA.

Kociba, R.J., D.G. Keyes, J.E. Beyer, et al. 1978a. Results of a two-year chronic toxicity and oncogenicity study of 2,3,7,8-tetrachlorodibenzo-p-dioxin in rats. Toxicol. Appl. Pharmacol. 46(2): 279-303.

Kociba, R.J., D.G. Keyes, J.E. Beyer and R.M. Carreon. 1978b. Toxicologic studies of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in rats. Toxicol. Occup. Med. (Dev. Toxicol. Environ. Sci.) 4: 281-287.

Kociba, R.J., D.G. Keyes, J.E. Beyer, R.M. Carreon and P.J. Gehring. 1979. Long-term toxicologic studies of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in laboratory animals. Ann. NY Acad. Sci. 320: 397-404.

Kohli, K.K. and J.A. Goldstein. 1981. Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on hepatic and renal prostaglandin synthetase. Life Sci. 29(3): 299-305.

Kondorosi, A., I. Fedorcsak, F. Solymosy, L. Ehrenberg and S. Osterman-Golkar. 1973. Inactivation of QBRNA by electrophiles. Mutat. Res. 17: 149-161.

Kooke, R., J.W.A. Lustenhouwer, K. Olie and O. Hutzinger. 1981. Extraction efficiencies of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans from fly ash. Anal. Chem. 53: 461-463.

Korfmacher, W.A., K.R. Rowland, R.K. Mitchum, J.J. Daly, R.C. McDaniel and M.V. Plummer. 1984. Analysis of snake tissue and snake eggs from 2,3,7,8-tetrachlorodibenzo-p-dioxin via fused silica GC combined with atmospheric pressure ionization MS. Chemosphere. 13: 1229-1233.

Kouri, R. 1976. Relationship between levels of aryl hydrocarbon hydroxylase activity and susceptibility to 3-methylcholanthrene and benzo(a)pyrene-induced cancers in inbred strains of mice. In: Polynuclear Aromatic Hydrocarbons: Chemistry, Metabolism and Carcinogenesis, Vol. 1, R.J. Freudenthal and P.W. Jones, Ed. Raven Press, NY. p. 139.

Kouri, R.E., H. Ratrie, III, S.A. Atlas, A. Niwa and D.W. Nebert. 1974. Aryl hydrocarbon hydroxylase induction in human lymphocyte cultures by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Life Sci. 15(9): 1585-1595.

Kouri, R.E., T.H. Rude, R. Joglekar, et al. 1978. 2,3,7,8-Tetrachlorodibenzo-p-dioxin as cocarcinogen causing 3-methylcholanthrene-initiated subcutaneous tumors in mice genetically "nonresponsive" at Ah locus. Cancer Res. 38(9): 2777-2783.

Kurl, R.N., J. Lund, L. Poellinger and J.A. Gustafsson. 1982. Differential effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin on nuclear RNA polymerase activity in the rat liver and thymus. Biochem. Pharmacol. 31(15): 2459-2462.

Kuroki, H. and Y. Masuda. 1977. Structures and concentrations of the main components of polychlorinated biphenyls retained in patients with Yusho. Chemosphere. 6: 465-469.

Lamb, J.C., J.A. Moore and T.A. Markes. 1980. Evaluation of 2,4-D, 2,4,5-T, and TCDD toxicity in C57BL/6 mice: Reproduction and fertility in treated male mice and evaluation of congenital malformation in their offspring. National Toxicology Program, Research Triangle Park, NC.

Lamb, J.C., T.A. Markes, B.C. Gladen, J.W. Allen and J.A. Moore. 1981a. Male fertility, sister chromatid exchange, and germ cell toxicity following exposure to mixtures of chlorinated phenoxy acids containing 2,3,7,8-tetrachlorodibenzo-p-dioxin. J. Toxicol. Environ. Health. 8: 825-834.

Lamb, J.C., J.A. Moore, T.A. Marks and J.K. Haseman. 1981b. Development and viability of offspring of male mice treated with chlorinated phenoxy acids and 2,3,7,8-tetrachlorodibenzo-p-dioxin. J. Toxicol. Environ. Health. 8(5-6): 835-844.

Lamparski, L.L. and T.J. Nestrick. 1980. Determination of tetra-, hexa-, hepta-, and octachlorodibenzo-p-dioxin isomers in particulate samples at parts per trillion levels. Anal. Chem. 52: 2045-2054.

Lamparski, L.L., N.H. Mahle and L.A. Shadoff. 1978. Determination of pentachlorophenol, hexachlorodibenzo-p-dioxin, and octachlorodibenzo-p-dioxin in bovine milk. J. Agric. Food Chem. 26(5): 1113-1116.

Lamparski, L.L., T.J. Nestrick and R.H. Stehl. 1979. Determination of part-per-trillion concentrations of 2,3,7,8-tetrachlorodibenzo-p-dioxin in fish. Anal. Chem. 51(9): 1453-1458.

Lamparski, L.L., R.L. Stehl and R.L Johnson. 1980. Photolysis of pentachlorophenol-treated wood; chlorinated dibenzo-p-dioxin formation. J. Environ. Sci. Tech. 14: 196-200.

Langhorst, M.L. and L.A. Shadoff. 1980. Determination of part-per-trillion concentrations of tetra-, hexa-, hepta-, and octa-chlorodibenzo-p-dioxins in human milk samples. Anal. Chem. 52: 2037-2044.

Lathrop, G.D., P.M. Moynahan, R.A. Albanese and W.D. Wolfe. 1983. An epidemiologic investigation of health effects in Air Force personnel following exposure to herbicides. Baseline mortality study results. June 30. USAF School of Aerospace Medicine (AFSC), Brooks Air Force Base, TX.

Lathrop, G.D., W.D. Wolfe, R.A. Albanese and P.M. Moynahan. 1984. An epidemiologic investigation of health effects in Air Force personnel following exposure to herbicides. Executive summary baseline morbidity study. In: Biological Mechanisms of Dioxin Action. Banbury Report 18. A. Poland and R.D. Kimbrough, Ed. Cold Spring Harbor Laboratory. p. 471-474.

Lavy, T.L., J.S. Shepard and J.D. Mattice. 1980. Exposure measurements of applicators spraying (2,4,5-trichlorophenoxy) acetic acid in the forest. J. Agric. Food Chem. 28: 626-630.

Lee, I.P and K. Suzuki. 1980. Induction of aryl hydrocarbon hydroxylase activity in the rat prostate glands by 2,3,7,8-tetrchlorodibenzo-p-dioxins. J. Pharmaccl. Exp. Ther. 215(3): 601-605.

Legraverend, C., B. Mansour, D.W. Nebert and J.M. Holland. 1980. Genetic differences in benzo[a]pyrene-initiated tumorigenesis in mouse skin. Pharmacology. 20: 242-255.

Leo, A.J. 1979. Letter to C. Stephan. Pamona College, Claremont, CA. May 11.

Levin, J.O., C.F. Rappe and C.A. Nilssen. 1976. Use of chlorophenols or fungicides in sawmills. Scand. J. Work Environ. Health. 2: 71.

Liberti, A. and D. Brocco. 1981. Formation of polychlorodibenzodioxins in urban incinerator emissions. In: Impact of Chlorinated Dioxins and Related Compounds on the Environment, O. Hutzinger et al., Ed. Pergamon Press, Oxford. p. 245-251.

Liem, H.H., U. Muller-Eberhard and E.F. Johnson. 1980. Differential induction by 2,3,7,8-tetrachlorodibenzo-p-dioxin of multiple forms of rabbit microsomal cytochrome P-450: Evidence for tissue specificity. J. Mol. Pharmacol. 18(3): 565-570.

Lindahl, R., M. Roper and R.A. Dietrich. 1978. Rat liver aldehyde dehydrogenase -- Immunochemical identity of 2,3,7,8-tetrachlorodibenzo-p-dioxin inducible normal liver and 2-acetylaminofluorene inducible hepatoma isozymes. Biochem. Pharmacol. 27(20): 2463-2465.

Liss, Q.S. and P.G. Slater. 1974. Flux of gases across the air-sea interface. Nature. 247: 181.

Loprieno, N., I. Sbrana, D. Rusciano, D. Lascialfari and T. Lari. 1982. In vivo cytogenetic studies on mice and rats exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin. In: Chlorinated Dioxins and Related Compounds. Impact on the Environment, O. Hutzinger, R.W. Frei, E. Merian and P. Pocchiari, Ed. Pergamon Press, NY. p. 419-428.

Lustenhouwer, J.W.A., K. Olie and O. Hutzinger. 1980. Chlorinated dibenzo-p-dioxins and related compounds in incinerator effluents: A review of measurements and mechanisms of formation. Chemosphere. 9: 501-522.

Luster, M.I., R.E. Faith and G. Clark. 1979a. Laboratory studies on the immune effects of halogenated aromatics. Ann. NY Acad. Sci. 320: 473-484.

Luster, M.I., G. Clark, L.D. Lawson and R.E. Faith. 1979b. Effects of a brief in vitro exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on mouse lymphocytes. J. Environ. Pathol. Toxicol. 2(4): 965-977.

Luster, M.I., G.A. Boorman, J.H. Dean, et al. 1980. Examination of bone marrow, immunologic parameters and host susceptibility following pre- and postnatal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Int. J. Immunopharmacol. 2(4): 301-310.

Mabey, W.R., J.H. Smith, R.T. Podoll, et al. 1981. Aquatic Fate Processes Data for Organic Priority Pollutants. EPA 440/4-81-014, Monitoring and Data Support Div., Office of Water and Regulations and Standards, Washington, DC. p. 107-108.

Madge, D.S. 1977. Effects of trichlorophenoxyacetic acid and chlorodioxins on small intestinal function. Gen. Pharmacol. 8: 319-324.

Mahle, N.H. and L.A. Shadoff. 1982. The mass spectrometry of chlorinated dibenzo-p-dioxins. Biomed. Mass Spectrom. 9: 45-60.

Mahle, N.H., H.S. Higgins and M.E. Getzendaner. 1977. Search for the presence of 2,3,7,8-tetrachlorodibenzo-p-dioxin in bovine milk. Bull. Environ. Contam. Toxicol. 18(2): 123-130.

Malik, N. and I.S. Owens. 1977. Studies on the requirements for induction of UDP glucuronosyl-transferase (T'ASE) activity in the Reuber hepatoma H-4-II-E established cell line (Meeting Abstract). Pharmacology. 19(2): 150.

Malik, N., G.M. Koteen and I.S. Owens. 1979. Induction of UDP-glucuronosyl-transferase activity in the Reuber H-4-II-E hepatoma cell culture. Mol. Pharmacol. 16(3): 950-960.

Manara, L., P. Coccia and T. Croci. 1982. Persistent tissue levels of TCDD in the mouse and their reduction as related to prevention of toxicity. Drug Metab. Rev. 13(3): 423-446.

Manis, J. and R. Apap. 1979. Intestinal organic anion transport, glutathione transferase and aryl hydrocarbon hydroxylase activity: Effect of dioxin. Life Sci. 24(15): 1373-1380.

Manis, J. and G. Kim. 1979a. Stimulation of iron absorption by polychlorinated aromatic hydrocarbons. Am. J. Physiol. 236(6): E763-E768.

Manis, J. and G. Kim. 1979b. Introduction of iron transport by a potent inducer of aryl hydrocarbon hydroxylase, 2,3,7,8-tetrachlorodibenzo-p-dioxin. Arch. Environ. Health. 34(3): 141-145.

Mantel, N. and M.A. Schneiderman. 1977. Estimation of "safe" levels, a hazardous undertaking? Cancer Res. 35: 1379-1386.

Mantovani, A., A. Vecchi, W. Luini, et al. 1980. Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin on macrophase and natural killer cell-mediated cytotoxicity in mice. Biomedicine. 32(4): 200-204.

Marks, T.A., G.A. Kimmel and R.E. Staples. 1981. Influence of symmetrical polychlorinated biphenyl isomers on embryo and fetal development in mice. Toxicol. Appl. Pharmacol. 61: 269-276.

Marselos, M., R. Torronen and A. Aitio. 1978. Responses of the D-glucuronic acid pathway in rat tissues to treatment with tetrachlorodibenzo dioxin. Xenobiotics. 8(7): 397-402.

Mason, M.E. and A.B. Okey. 1982. Cytosolic and nuclear binding of 2,3,7,8-tetrachlorodibenzo-p-dioxin to the AH receptor in extra-hepatic tissues of rats and mice. Eur. J. Biochem. 123(1): 209-215.

Matsumura, F. and H.J. Benezet. 1973. Studies on the bioaccumulation and microbial degradation of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Environ. Health Perspect. 5: 253-258.

Matsumura, F., J. Quensen and G. Tsushimoto. 1983. Microbial degradation of TCDD in a model ecosystem. In: Human and Environmental Risks of Chlorinated Dioxins and Related Compounds, R.E. Tucker, A.L. Young and A.P. Gray, Ed. Plenum Press, NY. p. 191-219.

Mattison, D.R. and S.S. Thorgeirsson. 1978. Gonadal aryl hydrocarbon hydroxylase in rats and mice. Cancer Res. 38(5): 1368-1373.

Mattison, D.R. and S.S. Thorgeirsson. 1979. Ovarian aryl hydrocarbon hydroxylase activity and primordial oocyte toxicity of polycyclic aromatic hydrocarbons in mice. Cancer Res. 39: 3471-3475.

Mattison, D.R., M.S. Nigthingale and E.K. Silbergeld. 1984. Reproductive toxicity of tetrachlorodibenzo-p-dioxin. In: Public Health Risks of the Dioxins, W.W. Lawrence, Ed. William Kaufmann, Los Altos, CA. p. 217-235.

May, G. 1973. Chloracne from the accidental production of tetrachlorodibenzodioxin. Br. J. Ind. Med. 30(3): 276-283.

May, G. 1982. Tetrachlorodibenzodioxin: A survey of subjects ten years after exposure. Br. J. Ind. Med. 39(2): 128-135.

McCann. 1978. Unpublished study. (Cited in Wassom et al., 1978)

McConnell, E.E. 1980. Acute and chronic toxicity, carcinogenesis, reproduction, teratogenesis and mutagenesis in animals. In: Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins and Related Products, R.D. Kimbrough, Ed. Elsevier/North-Holland Biomedical Press, NY. p. 109-150.

McConnell, E.E. and J.A. Moore. 1979. Toxicopathology characteristics of the halogenated aromatics. Ann. NY Acad. Sci. 320: 138-150.

McConnell, E.E., J.A. Moore and D.W. Dalgard. 1978a. Toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in rhesus monkeys (Macacas mulatta) following a single oral dose. Toxicol. Appl. Pharmacol. 43: 175.

McConnell, E.E., J.A. Moore, J.K. Haseman and M.W. Harris. 1978b. The comparative toxicity of chlorinated dibenzo-p-dioxins in mice and guinea pigs. Toxicol. Appl. Pharmacol. 44(2): 335-356.

McConnell, E.E., G.W. Lucier, R.C. Rumbaugh, et al. 1984. Dioxin in soil: Bioavailability after ingestion by rats and guinea pigs. Science. 223: 1077-1079.

McGaughy, R. and A. Rispin. 1985. U.S. EPA, Washington, DC. Memorandum to D. Barns, U.S. EPA, Washington, DC, August 8.

McKinney, J.D., K. Chae, B.N. Gupta, J.A. Moore and J.A. Goldstein. 1976. Toxicological assessment of hexachlorobiphenyl isomers and 2,3,7,8-tetrachlorodibenzofuran in chicks. I. Relationship of chemical parameters. Toxicol. Appl. Pharmacol. 36: 65-80.

McKinney, J., P. Albro, M. Luster, B. Corbett, J. Schroeder and L. Lawson. 1981. Development and reliability of a radio immunoassay for 2,3,7,8-tetrachlorodibenzo-p-dioxin. In: Impact of Chlorinated Dioxins and Related Compounds on the Environment, O. Hutzinger et al., Ed. Pergamon Press, Oxford. p. 67-75.

McNulty, W.P. 1978. Direct testimony before the administrator, U.S. Environmental Protection Agency, FIFRA Docket No. 415, EPA Exhibit No. 106.

McNulty, W.P. 1984. Fetocidal and teragenic actions of TCDD. In: Public Health Risks of the Dioxins, W.W. Lawrence, Ed. William Kaufmann, Los Altos, CA. p. 245-253.

McNulty, W.P., K.A. Nielsen-Smith, J.O. Lay, Jr., et al. 1982. Persistence of TCDD in monkey adipose tissue. Food Cosmet. Toxicol. 20: 985-987.

McQueen, E.G., A.M.O. Veale, W.S. Alexander and M.N. Bates. 1977. 2,4,5-T and human birth defects. Report of the Division of Public Health, New Zealand Dept. of Health. (Cited in Milby et al., 1980)

Meselson, M., P.W. O'Keefe and R. Baughman. 1978. The evaluation of possible health hazards from TCDD in the environment. Presented at the Symposium on the Use of Herbicides in Forestry, Arlington, VA. February 21-22.

Michigan Department of Public Health. 1983a. Evaluation of Congenital Malformation Rates for Midland and Other Selected Michigan Counties Compared Nationally and Statewise 1970-1981. Michigan Dept. of Public Health. May 4.

Michigan Department of Public Health. 1983b. Evaluation of Soft and Connective Tissue Cancer Mortality Rates for Midland and Other Selected Michigan Counties Compared Nationally and Statewide. Michigan Dept. of Public Health. May 4.

Miettinen, O. 1976. Estimability and estimation in case referent studies. Am. J. Epidemiol. 103: 226.

Milby, T.H., E.L. Husting, M.D. Whorton and S. Larson. 1980. Potential Health Effects Associated with the Use of Phenoxy Herbicides: A Summary of Recent Scientific Literature. A Report for the National Forest Products Association from Environmental Health Associated, Inc., Berkeley, CA.

Miller, R.A., L.A. Norris and C.L. Hawkes. 1973. Toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in aquatic organisms. Environ. Health Perspect. 5: 177-186.

Miller, R.A., L.A. Norris and B.R. Loper. 1979. The response of coho salmon and guppies to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in water. Am. Fish. Soc. Trans. 108(4): 401-407.

Mitchum, R.K., G.F. Moler and W.A. Korfmacher. 1980. Combined capillary gas chromatography/atmospheric pressure negative chemical ionization/mass spectrometry for the determination of 2,3,7,8-tetrachlorodibenzo-p-dioxin in tissue. Anal. Chem. 52(14): 2278-2282.

Moore, J.A. and R.E. Faith. 1976. Immunologic response and factors affecting its assessment. Environ. Health Perspect. 18: 125-131.

Moore, J.A. and J.G. Vos. 1974. Evaluating the rodent immune system as a focus for tetratogenic effects. Teratology. 9(3): A-29.

Moore, J.A., B.N. Gupta, J.G. Zinkl and J.G. Vos. 1973. Postnatal effects of maternal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Environ. Health Perspect. 5: 81-85.

Moore, J.A., M.W. Harris and P.W. Albro. 1976. Tissue distribution of ($^{14}$C)tetrachlorodibenzo-p-dioxin in pregnant and neonatal rats. Toxicol. Appl. Pharmacol. 37(1): 146-147.

Moore, J.A., E.E. McConnell, D.W. Dalgard and M.W. Harris. 1979. Comparative toxicity of three halogenated dibenzofurans in guinea pigs, mice and rhesus monkeys. Ann. NY. Acad. Sci. 320: 151-163.

Moore, T. and I.M. Sharman. 1950. Vitamin A in the kidney of male and female rats. Biochem. J. 47: xliii-xliv.

Mortelmans, K., S. Haworth, W. Speck and E. Zieger. 1984. Mutagenicity testing of Agent Orange components and related compounds. Toxicol. Appl. Pharmacol. 75: 137-146.

Moses, M. and I.J. Selikoff. 1981. Soft-tissue sarcomas, phenoxy herbicides and chlorinated phenols. Lancet. 1(8234): 1370.

Mottura, A., G. Zei, F. Nuzzo, et al. 1981. Evaluation of results of chromosome analyses on lymphocytes of TCDD exposed subjects after the Seveso accident. Mutat. Res. 85(4): 238-239.

Mulcahy, M.T. 1980. Chromosome aberrations and "Agent Orange". Med. J. Aust. 2(10): 573-574.

Murray, F.J., F.A. Smith, K.D. Nitschke, C.G. Humiston, R.J. Kociba and B.A. Schwetz. 1979. Three-generation reproduction study of rats given 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in the diet. Toxicol. Appl. Pharmacol. 50: 241-251.

Muscarella, D., D. Dennett, J.G. Babish and D. Noden. 1982. The effects of 2,3,7,8-tetrachlorodibenzo-p-dioxins on fetal development in the ferret. Toxicologist. 2: 73.

Nagarkatti, P.S., G.D. Sweeney, J. Gauldie and D.A. Clark. 1984. Sensitivity to suppression of cytotoxic T cell generation by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is dependent on the AH genotype of the murine host. Toxicol. Appl. Pharmacol. 72: 169-176.

NAS (National Academy of Sciences). 1977. Drinking Water and Health: Part II. NAS, Washington, DC.

Nash, R.G. and M.L. Beall, Jr. 1980. Distribution of Silvex, 2,4-D, and TCDD applied to turf in chambers and field plots. J. Agric. Food Chem. 28: 614-623.

Nau, H. and R. Bass. 1981. Transfer of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) to the mouse embryo and fetus. Toxicology. 20(4): 299-308.

Nau, H., R. Bass and D. Neubert. 1982. Transfer of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) to the mouse embryo, fetus and neonate. In: Chlorinated Dioxins and Related Compounds. Impact on the Environment, O. Hutzinger, R.W. Frei, E. Merian and F. Pocchiari, Ed. Pergamon Press, NY. p. 325-337.

Neal, R.A., P.W. Beatty and T.A. Gasiewicz. 1979. Studies of the mechanisms of toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Ann. NY Acad. Sci. 320: 204-213.

Neal, R.A., J.R. Olson, T.A. Gasiewicz and L.E. Geiger. 1982. The toxicokinetics of 2,3,7,8-tetrachlorodibenzo-p-dioxin in mammalian systems. Drug Metab. Rev. 13: 355-385.

Neal, R.A., T. Gasiewicz, L. Geiger, J. Olson and T. Sawahata. 1984. Metabolism of 2,3,7,8-tetrachlorodibenzo-p-dioxin in mammalian systems. In: Biological Mechanisms of Dioxin Action. Banbury Report 18. A. Poland and R.D. Kimbrough, Ed. Cold Spring Harbor Laboratory. p. 49-60.

Nebert, D.W. 1979. Multiple forms of inducible drug-metabolizing enzymes: A reasonable mechanism by which any organism can cope with adversity. Mol. Cell. Biochem. 27: 27-46.

Nebert, D.W. 1980. The Ah locus. A gene with possible importance in cancer predictability. Quantitative aspects of risk assessment in chemical carcinogenesis. Arch. Toxicol. (Suppl. 3): 195-207.

Nebert, D.W. 1982. Pharmacogenetics and human cancer. Host factors in human carcinogenesis. IARC Scientific Pub. No. 39. Lyon, France.

Nebert, D.W. and J.E. Gielen. 1972. Genetic regulation of aryl hydrocarbon hydroxylase induction in the mouse. Fed. Proc. 31: 1315-1327.

Nebert, D.W. and N.M. Jensen. 1979. The Ah locus: Genetic regulation of the metabolism of carcinogens, drugs, and other environmental chemicals by cytochrome P-450 mediated monooxygenases. CRC Crit. Rev. Biochem. 6: 401-437.

Nebert, D.W., F.W. Goujon and J.E. Gielen. 1972. Aryl hydrocarbon hydroxylase induction by polycyclic hydrocarbons: Simple autosomal dominant trait in the mouse. Natl. New. Biol. 236: 107-110.

Nebert, D.W., et al. 1975. Genetic expression of aryl hydrocarbon hydroxylase activity in the mouse. J. Cell. Physiol. 85: 393-414.

Nebert, D., S. Thorgiersson and J. Felton. 1976. Genetic differences in mutagenesis, carcinogenesis, and drug toxicity. In: In vitro Metabolic Activation in Mutagenesis Testing, F. de Serres, J. Folets, J. Bend and R. Philpot, Ed. Elsevier/North Holland Biomedical Press, Amsterdam. p. 105-124.

Nebert, D.W., H.J. Eisen, M. Negishi, M.A. Lang, L.M. Hjelmeland and A.B. Okey. 1981. Genetic mechanisms controlling the induction of polysubstrate monooxygenase (P-450) activities. Ann. Rev. Pharmacol. Toxicol. 21: 431-462.

Nebert, D.W., M. Negishi and H.J. Eisen. 1983. Genetic differences in enzymes which metabolize drugs, chemical carcinogens and other environmental pollutants. In: Human and Environmental Risks of Chlorinated Dioxins and Related Compounds, R.E. Rucker, A.L. Young and A.P. Gray, Ed. Plenum Press, NY. p. 441-462.

Neely, W.B. 1979. Estimating rate constants for the uptake and clearance of chemicals by fish. Environ. Sci. Technol. 13: 1506.

Neely, W.B. 1983. Letter to C.E. Stephan. Dow Chemical Co., Midland, MI. June 10.

Nelson, C.J., J.F. Holson, H.G. Green and D.W. Gaylor. 1979. Retrospective study of the relationship between agricultural use of 2,4,5-T and cleft palate occurrence in Arkansas. Teratology. 19: 377-384.

Nelson, J.O., R.E. Menzer, P.C. Kearney and J.R. Plimmer. 1977. 2,3,7,8-Tetrachlorodibenzo-p-dioxin: In vitro binding to rat liver microsomes. Bull. Environ. Contam. Toxicol. 18(1): 9-13.

Nestrick, T.J., L.L. Lamparski and D.I. Townsend. 1980. Identification of tetrachlorodibenzo-p-dioxin isomers at the 1 ng level by photolytic degradation and pattern recognition techniques. Anal. Chem. 52(12): 1865-1874.

Nestrick, T.J., L.L. Lamparski, W.B. Crummet and L.A. Shadoff. 1982. Comments on variations in concentrations of organic compounds including polychlorinated dibenzo-p-dioxins and polynuclear aromatic hydrocarbons in fly ash from a municipal incinerator. Anal. Chem. 54: 823-824.

Neubert, D. and I. Dillmann. 1972. Embryotoxic effects in mice treated with 2,4,5-trichlorophenoxyacetic acid and 2,3,7,8-tetrachlorodibenzo-p-dioxin. Arch. Pharmacol. 272(3): 243-264.

Neubert, D., P. Zens, A. Rothenwallner and H.J. Merker. 1973. A survey of the embryotoxic effects of TCDD in mammalian species. Environ. Health Perspect. 5: 67-79.

Niemann, R.A., W.C. Brumley, D. Firestone and J.A. Sphon. 1983. Analysis of fish for 2,3,7,8-tetrachlorodibenzo-p-dioxin by electron capture gas chromatography. Anal. Chem. 55: 1497-1504.

Nienstedt, W., M. Parkki, P. Uotila and A. Aitio. 1979. Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on hepatic metabolism of testosterone in the rat. Toxicology. 13(3): 233-236.

Nilsson, C.-A., K. Andersson, C. Rappe and S.-O. Westermark. 1974. Chromatographic evidence for the formation of chlorodioxins from chloro-2-phenoxyphenols. J. Chromatogr. 96: 137-147.

NIOSH (National Institute for Occupational Safety and Health). 1982. Health Hazard Evaluation Determination Report: Long Island Railroad. No. 80-039.

Nisbet, I.C.T. and M.B. Paxton. 1982. Statistical aspects of three-generation studies of the reproductive toxicity of TCDD and 2,4,5-T. Am. Stat. Vol. 36(3): 290-298.

Niwa, A., K. Kumaki and D.W. Nebert. 1975. Induction of aryl hydrocarbon hydroxylase activity in various cell cultures by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Mol. Pharmacol. 11(4): 399-408.

Nolan, R.J., F.A. Smith and J.G. Hefner. 1979. Elimination and tissue distribution of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in female guinea pigs following a single oral dose. Toxicol. Appl. Pharmacol. 48(1): A162.

Norback, D.H. and J.R. Allen. 1973. Biological responses of the nonhuman primate, chicken, and rat to chlorinated dibenzo-p-dioxin ingestion. Environ. Health Perspect. 5: 233-240.

Norman, R.L., E.F. Johnson and U. Muller-Eberhard. 1978a. Identification of the major cytochrome P-450 form transplacentally induced in neonatal rabbits by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Fed. Proc. Am. Soc. Exp. Biol. 37(6): 1720.

Norman, R.L., E.F. Johnson and U. Muller-Eberhard. 1978b. Identification of the major cytochrome P-450 form transplacentally induced in neonatal rabbits by 2,3,7,8-tetrachlorodibenzo-p-dioxin. J. Biol. Chem. 253(23): 8640-8647.

Norris, L.A. and R.A. Miller. 1974. The toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in guppies (Poecilia reticulatus Peters). Bull. Environ. Contam. Toxicol. 12(1): 76-80.

Norstrom, A., C. Rappe, R. Lindahl and H.R. Buser. 1979. Analysis of some older Scandinavian formulations of 2,4-dichlorophenoxy acetic acid and 2,4,5-trichlorophenoxy acetic acid for contents of chlorinated dibenzo-p-dioxins and dibenzofurans. Scand. J. Work Environ. Health. 5(4): 375-378.

Norstrom, R.J., D.J. Hallett, M. Simon and M.J. Mulvihill. 1982. Analysis of Great Lakes herring gull eggs for tetrachlorodibenzo-p-dioxins. In: Chlorinated Dioxins and Related Compounds. Impact on the Environment, O. Hutzinger, R.W. Frei, E. Merian and E. Pocchiari, Ed. Pergamon Press, NY. p. 173-181.

NRCC (National Research Council of Canada). 1981a. Polychlorinated Dibenzo-p-Dioxins: Criteria for their Effects on Man and His Environment. Publ. No. NRCC 18574, ISSN 0316-0114. NRCC/CNRC Associate Committee on Scientific Criteria for Environmental Quality, Ottawa, Canada. 251 p.

NRCC (National Research Council of Canada). 1981b. Polychlorinated Dibenzo-p-Dioxins: Limitations to the Current Analytical Techniques. Publ. No. 18576, NRCC/NRC, Ottawa, Canada.

NTP (National Toxicology Program). 1980a. Bioassay of 2,3,7,8-tetrachlorodibenzo-p-dioxin for possible carcinogenicity (gavage study). DHHS Publ. No. (NIH) 82-1765. Carcinogenesis Testing Program, NCI, NIH, Bethesda, MD, and National Toxicology Program, RTP, Box 12233, NC.

NTP (National Toxicology Program). 1980b. Bioassay of 2,3,7,8-tetrachlorodibenzo-p-dioxin for possible carcinogenicity (Dermal study). DHHS Publ. No. (NIH) 80-1757. Carcinogenesis Testing Program, NCI, NIH, Bethesda, MD, and National Toxicology Program, RTP, Box 12233, NC.

NTP (National Toxicology Program). 1980c. Bioassay of 1,2,3,6,7,8- and 1,2,3,7,8,9-hexachlorodibenzo-p-dioxin for possible carcinogenicity (dermal study). DHHS Publ. No. (NIH) 80-1758. Carcinogenesis Testing Program, NCI, NIH, Bethesda, MD, and National Toxicology Program, NTP, Box 12233, NC.

NTP (National Toxicology Program). 1980d. Bioassay of 1,2,3,6,7,8- and 1,2,3,7,8,9-hexachlorodibenzo-p-dioxin (gavage) for possible carcinogenicity. DHHS Publ. No. (NIH) 80-1754. Carcinogenesis Testing Program, NCI, NIH, Bethesda, MD, and National Toxicology Program, RTP, Box 12233, NC.

NTP (National Toxicology Program). 1985. Unpublished results. Personal communication from Beth Tainer, NIEHS, Research Triangle Park, NC to Dr. D. Mukerjee, ECAO, U.S. EPA, Cincinnati, OH. March 18.

Ogilvie, D. 1981. Dioxin found in the Great Lakes basin. Ambio. 10: 38-39.

O'Keefe, P.W., M.S. Meselson and R.W. Baughman. 1978. Neutral cleanup procedure for 2,3,7,8-tetrchlorodibenzo-p-dioxin residues in bovine fat and milk. J. Assoc. Off. Anal. Chem. 61(3): 621-626.

O'Keefe, P., C. Meyer and K. Dillon. 1982. Comparison of concentration techniques for 2,3,7,8-tetrachlorodibenzo-p-dioxin. Anal. Chem. 54: 2623-2625.

O'Keefe, P., C. Meyer, D. Hilker, et al. 1983. Analysis of 2,3,7,8-tetrachlorodibenzo-p-dioxin in Great Lakes fish. Chemosphere. 12: 325-332.

Okey, A.G. 1983. The Ah receptor: A specific site for action of chlorinated dioxins. In: Human and Environmental Risks of Chlorinated Dioxins and Related Compounds, R.E. Tucker, A.L. Young and A.P. Gray, Ed. Plenum Press, NY. p. 423-440.

Okey, A.B. and L.M. Vella. 1982. Binding of 3-methylcholanthrene and 2,3,7,8-tetrachlorodibenzo-p-dioxin to a common Ah receptor site in mouse and rat hepatic cytosis. Eur. J. Biochem. 127(1): 39-47.

Okey, A.B. and L.M. Vella. 1984. Elevated binding of 2,3,7,8-tetrachlorodibenzo-p-dioxin and 3-methylcholanthrene to the Ah receptor in hepatic cytosols from phenobarbital-treated rats and mice. Biochem. Pharmacol. (In press)

Okey, A.B., G.P. Bondy, M.E. Mason, et al. 1979. Regulatory gene product of the Ah locus. J. Biol. Chem. 254: 11636-11648.

Okey, A.B., G.P. Bondy, M.E. Mason, et al. 1980. Temperature-dependent cytosol-to-nucleus translocation of the Ah receptor for 2,3,7,8-tetrachlorodibenzo-p-dioxin in continuous cell culture lines. J. Biol. Chem. 255(23): 11415-11422.

Olie, K., J.W.A. Lustenhouwer and O. Hutzinger. 1982. Polychlorinated dibenzo-p-dioxins and related compounds in incinerator effluents. In: Chlorinated Dioxins and Related Compounds: Impact on the Environment, O. Hutzinger et al., Ed. Pergamon Press, NY. p. 227-244.

Olie, K., M.V.D. Berg and O. Hutzinger. 1983. Formation and fate of PCDD and PCDF from combustion processes. Chemosphere. 12: 627-636.

Oliver, J.E. and J.M. Ruth. 1983. Nitration of two TCDDs and their conversion to 1,2,3,6,7,8-HCDD. Chemosphere. 12: 1497-1503.

Oliver, R.M. 1975. Toxic effects of 2,3,7,8-tetrachlorodibenzo-1,4-dioxin in laboratory workers. Br. J. Ind. Med. 32(1): 49-53.

Olson, J.R. and W.E. Bittner. 1983. Comparative metabolism and elimination of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Toxicologist. 3: 103.

Olson, J.R., T.A. Gasiewicz and R.A. Neal. 1980a. Tissue distribution, excretion, and metabolism of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in the Golden Syrian hamster. Toxicol. Appl. Pharmacol. 56: 78-85.

Olson, J.R., M.A. Holscher and R.A. Neal. 1980b. Toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the Golden Syrian hamster. Toxicol. Appl. Pharmacol. 55: 67-78.

Olson, J.R., M. Gudzinowicz and R.A. Neal. 1981. The in vitro and in vivo metabolism of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in the rat. Toxicologist. 1: 69-70.

Olson, J.R., Gasiewicz, T.A., L.E. Geiger and R.A. Neal. 1983. The metabolism of 2,3,7,8-tetrachlorodibenzo-p-dioxin in mammalian systems. In: Accidental Exposure to Dioxins: Human Health Aspects, F. Coulston and F. Pocchiari, Ed. Academic Press, NY. p. 81-100.

Olsson, H. and L. Brandt. 1981. Non-Hodgkin's lymphoma of the skin and occupational exposure to herbicides. Lancet. 9/12/81: 579.

Orris, P. 1981. Unjustified conclusion? J. Occup. Med. 23(1): 7-8.

Ott, M.G., B.B. Holder and R.D. Olson. 1980. A mortality analysis of employees engaged in the manufacture of 2,4,5-trichlorophenoxyacetic acid. J. Occup. Med. 22(1): 47-50.

Owens, I.S. 1977. Genetic regulation of UDP-glucuronosyltranferase induction by polycyclic aromatic compounds in mice. J. Biol. Chem. 252: 2827-2833.

Parker, C.E., W.A. Jones, H.B. Matthews, E.E. McConnell and J.R. Hass. 1980. Chronic toxicity of technical and analytical pentachlorophenol in cattle. II. Chemical analysis of tissues. Toxicol. Appl. Pharmacol. 55(2): 359-369.

Parkinson, A., R. Cockerline and S. Safe. 1980a. Polychlorinated biphenyl isomers and congeners as inducers of both 3-methylcholanthrene- and phenobarbitone-type microsomal enzyme activity. Chem.-Biol. INteract. 29: 277-289.

Parkinson, A., L. Robertson, L. Safe and S. Safe. 1980b. Polychlorinated biphenyls as inducers of hepatic microsomal enzymes: Structure-activity rules. Chem.-Biol. Interact. 30: 271-285.

Parkinson, A., R. Cockerline and S. Safe. 1980c. Induction of both 3-methylcholanthrene- and phenobarbitone-type microsomal enzyme activity by a single polychlorinated biphenyl isomer. Biochem. Pharmacol. 29: 259-262.

Parkinson, A., L. Robertson, L. Uhlig, M.A. Campbell and S. Safe. 1982. 2,3,4,5',5-Pentachlorobiphenyl: Differential effects on C56BL/6J and DBA/2J inbred mice. Biochem. Pharmacol. 31: 2830-2833.

Parkinson, A., S. Safe, L. Robertson, et al. 1983. Immunochemical quantitation of cytochrome P-450 isozymes and epoxide hydrolase in liver microsomes from polychlorinated and polybrominated biphenyls: A study of structure activity relationships. J. Biol. Chem. 258: 5967-5976.

Passi, S., M. Nazzaro-Porro, L. Boniforti and F. Gianotti. 1981. Analysis of lipids and dioxin in chloracne due to tetrachloro-2,3,7,8-p-dibenzodioxin. Br. J. Dermatol. 105(2): 137-143.

Pazderova-Vejlupkova, J., M. Nemcova, J. Pickova, L. Jirasek and E. Lukas. 1981. The development and prognosis of chronic intoxication by tetrachlorodibenzo-p-dioxin in men. Arch. Environ. Health. 36(1): 5-11.

Pegg, D.G., W.R. Hewitt, K.M. McCormack and J.B. Hook. 1976. Effect of 2,3,7,8-tetrachlorodibenzo-rho-dioxin on renal function in the rat. J. Toxicol. Environ. Health. 2(1): 55-65.

Peterson, R.E., N. Hamada, K.H Yang, B.V. Madhukar and F. Matsumura. 1979a. Depression of adenosine triphosphatase activities in isolated liver surface membranes of 2,3,7,8-tetrachlorodibenzo-p-dioxin-treated rats: Correlation with effects on ouabain biliary excretion and bile flow. J. Pharmacol. Exp. Therap. 210(2): 275-282.

Peterson, R.E., N. Hamada, K.H. Yang, B.V. Madhukar and F. Matsumura. 1979b. Reversal of 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced depression of ouabain biliary excretion by pregnenolone-16alpha-carbonitrile and spironolactone in isolated perfused rat livers. Toxicol. Appl. Pharmacol. 50(3): 407-416.

Peterson, R.E., M.D. Seefeld, B.J. Christian, C.L. Potter, C.K. Kelling and R.E. Keesey. 1984. The wasting syndrome in 2,3,7,8-tetrachlorodibenzo-p-dioxin toxicity: Basic features and their interpretation. In: Biological Mechanisms of Dioxin Action. Banbury Report 18. A Poland and R.D. Kimbrough, Ed. Cold Spring Harbor Laboratory. p. 291-308.

Philippi, M., V. Krasnobagew, J. Zeyer and R. Huetter. 1981. Fate of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in microbial cultures and soil under laboratory conditions. FEMS Symp. 12: 2210-233.

Phillipson, D.W. and B.J. Puma. 1980. Identification of chlorinated methoxybiphenyls as contaminants in fish and as potential interferences in the determination of chlorinated dibenzo-p-dioxins. Anal. Chem. 42: 2328-2332.

Piper, W.N., R.Q. Rose and P.J. Gehring. 1973. Excretion and tissue distribution of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the rat. Environ. Health Perspect. 5: 241-244.

Pitot, H.C., T. Goldsworthy and H. Poland. 1980. Promotion by 2,3,7,8-tetrachlorodibenzo-p-dioxin of hepatocarcinogenesis from diethylnitrosamine. Cancer Res. 40: 3616-3620.

Plimmer, J.R. 1978. Photolysis of TCDD and trifluralin on silica and soil. Bull. Environ. Contam. Toxicol. 20: 87-92.

Plimmer, J.R., J.M. Ruth and E.A. Woolson. 1973. Mass spectrometric identification of the hepta- and octachlorinated dibenzo-p-dioxins and dibenzofurans in technical pentachlorophenol. J. Agric. Food Chem. 21(1): 90-93.

Pocchiari, F., V. Silano and A. Zampieri. 1979. Human health effects from accidental release of tetrachlorodibenzo-p-dioxin (TCDD) at Seveso, Italy. Ann. NY Acad. Sci. 320: 311-320.

Pocchiari, F., A. DiDomenico, V. Silano and G. Zapponi. 1983. Environmental impact of the accidental release of tetrachlorodibenzo-p-dioxin (TCDD) at Seveso (Italy). In: Accidental Exposure to Dioxins: Human Health Aspects, F. Coulston and F. Pocchiari, Ed. Academic Press, NY. p. 5-35.

Pohland, A.E. and G.C. Yang. 1972. Preparation and characterization of chlorinated dibenzo-p-dioxins. J. Agric. Food Chem. 20: 1093-1099.

Poiger, H. and C. Schlatter. 1979. Biological degradation of TCDD in rats. Nature. 281(5733): 706-707.

Poiger, H. and C. Schlatter. 1980. Influence of solvents and adsorbents on dermal and intestinal absorption of TCDD. Food Cosmet. Toxicol. 18(5): 477-481.

Poiger, H., H. Weber and C. Schlatter. 1982a. Special aspects of metabolism and kinetics of TCDD in dogs and rats. Assessment of toxicity of TCDD-metabolite(s) in guinea pigs. In: Chlorinated Dioxins and Related Compounds. Impact on the Environment, O. Hutzinger, R.W. Frei, E. Merian and F. Pocchiari, Ed. Pergamon Press, NY. p. 317-325.

Poiger, H., H.R. Buser, H. Weber, U. Zweifel and C. Schlatter. 1982b. Structure elucidation of mammalian TCDD-metabolites. Experientia. 38(4): 484-486.

Poland, A. 1984. Reflections on the mechanisms of action of halogenated aromatic hydrocarbons. In: Biological Mechanisms of Dioxin Action. Banbury Report 18. A. Poland and R.D. Kimbrough, Ed. Cold Spring Harbor Laboratory. p. 109-117.

Poland, A. and E. Glover. 1973a. Chlorinated dibenzo-p-dioxins: Potent inducers of δ-aminolevulinic acid synthetase and aryl hydrocarbon hydroxylase. II. A study of the structure activity relationship. Mol. Pharmacol. 9: 736.

Poland, A. and E. Glover. 1973b. 2,3,7,8-Tetrachlorodibenzo-p-dioxin. Potent inducer of delta-aminolevulinic acid synthetase. Science. 179(4072): 476-477.

Poland, A. and E. Glover. 1974. Comparison of 2,3,7,8-tetrachlorodibenzo-p-dioxin, a potent inducer of aryl hydrocarbon hydroxylase, with inducer 3-methylcholanthrene. Mol. Pharmacol. 10(2): 349-359.

Poland, A. and E. Glover. 1975. Genetic expression of aryl hydrocarbon hydroxylase by 2,3,7,8-tetrachlorodibenzo-p-dioxin: Evidence for a receptor mutation in genetically non-responsive mice. Mol. Pharmacol. 11(4): 389-398.

Poland, A. and E. Glover. 1979. An estimate of the maximum *in vivo* covalent binding of 2,3,7,8-tetrachlorodibenzo-p-dioxin to rat liver protein, ribosomal RNA and DNA. Cancer Res. 39(9): 3341-3344.

Poland, A. and E. Glover. 1980. 2,3,7,8-Tetrachlorodibenzo-p-dioxin: Segregation of toxicity with the Ah locus. Mol. Pharmacol. 17(1): 86-94.

Poland, A. and J.C. Knutson. 1982. 2,3,7,8-Tetrachlorodibenzo-p-dioxin and related halogenated aromatic hydrocarbons: Examination of the mechanism of toxicity. Ann. Rev. Pharmacol. Toxicol. 22: 517-554.

Poland, A.P., D. Smith, G. Metter and P. Possick. 1971. A health survey of workers in a 2,4-D and 2,4,5-T plant with special attention to chloracne, porphyria cutanea tarda, and psychologic parameters. Arch. Environ. Health. 22: 316-327.

Poland, A.P., E. Glover, J.R. Robinson and D.W. Nebert. 1974. Genetic expression of aryl hydrocarbon hydroxylase activity. Induction of monooxygenase activities and cytochrome $P_1$-450 formation by 2,3,7,8-tetrachlorodibenzo-p-dioxin in mice genetically "nonresponsive" to other aromatic hydrocarbons. J. Biol. Chem. 249(17): 5599-5606.

Poland, A., E. Glover and A.S. Kende. 1976. Stereospecific, high affinity binding of 2,3,7,8-tetrachlorodibenzo-p-dioxin by hepatic cytosol. Evidence that the binding species is a receptor for induction of aryl hydrocarbon hydroxylase. J. Biol. Chem. 251(16): 4936-4946.

Poland, A., W.F. Greenlee and A.S. Kende. 1979. Studies on the mechanisms of action of the chlorinated dibenzo-p-dioxins and related compounds. Ann. NY Acad. Sci. 320: 214-230.

Poland, A., D. Palen and E. Glover. 1982. Tumour promotion by TCDD in skin of HRS/J mice. Nature. 300(5889): 271-273.

Poland, A., J. Knutson and E. Glover. 1983. A consideration of the mechanism of action of 2,3,7,8-tetrachlorodibenzo-p-dioxin and related halogenated aromatic hydrocarbons. In: Human and Environmental Risks of Chlorinated Dioxins and Related Compounds, R.E. Rucker, A.L. Young and A.P. Gray, Ed. Plenum Press, NY. p. 539-559.

Poli, A., G. Grancescini, L. Puglisi and C.R. Sirtori. 1980. Increased total and high density lipoprotein cholesterol with apoprotein changes resembling streptozotocin diabetes in tetrachlorodibenzodioxin (TCDD) treated rats. Biochem. Pharmacol. 28(5): 835-838.

Poole, C. 1983. Statement of Charles Poole before the United States House of Representatives Science and Technology Committee, Subcommittee on Natural Resources, Agricultural Research and Environment. March 23.

Potter, C.L., I.G. Sipes and D.H. Russell. 1982. Inhibition of ornithine decarboxylase activity by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Biochem. Pharmacol. 31(21): 3367-3371.

Pratt, R.M. 1983. Mechanisms of chemically-induced cleft palate. Trends Pharmacol. Sci. 4: 160-162.

Pratt, R.M. and W.D. Willis. 1985. In vitro screening assay for teratogens using growth inhibition of human embryonic cells. Proc. Natl. Acad. Sci. USA. (In press, September issue)

Pratt, R.M., L. Dencker and V.M. Diewert. 1984a. TCCD-induced cleft palate in the mouse: Evidence for alterations in palatal shelf fusion. Teratol. Carcinog. Mutagen. 4: 427-436.

Pratt, R.M., R.I. Grove, C.S. Kim, L. Dencker and V.M. Diewert. 1984b. Mechanism of TCDD-induced cleft palate in the mouse. In: Biological Mechanisms of Dioxin Action. Banbury Report 18. A. Poland and R.D. Kimbrough, Ed. Cold Spring Harbor Laboratory. p. 61-71.

Ramsey, J.C., J.G. Hefner, R.J. Karbowski, W.H. Braun and P.J. Gehring. 1982. The in vitro biotransformation of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in the rat. Toxicol. Appl. Pharmacol. 65: 180-184.

Rappe, C., S. Marklund, H.R. Buser and H.P. Bosshardt. 1978. Formation of polychlorinated dibenzo-p-dioxins and dibenzofurans by burning or heating chlorophenates. Chemosphere. 7(3): 269-281.

Rappe, C., H.R. Buser and H.P. Bosshardt. 1979. Dioxins, dibenzofurans, and other polyhalogenated aromatics production use formation and destruction. Ann. NY Acad. Sci. 320: 1-18.

Rappe, C., S. Marklund, M. Nygren and A. Gari. 1983a. Parameter for identification and confirmation in trace analyses of polychlorinated dioxins and dibenzofurans. In: Chlorinated Dioxins and Dibenzofurans in the Total Environment, Vol. I, L.H. Keith et al., Ed. Butterworth Publishers.

Rappe, C., S. Marklund, P.A. Bergqvist and P. Hansson. 1983b. Polychlorinated dioxins, dibenzofurans and other polychlorinated polynuclear aromatics formed during incinerator and PCB fires. In: Chlorinated Dioxins and Dibenzofurans in the Total Environment, Vol. I, L.H. Keith et al., Ed. Butterworth Publishers.

Rappe, C., P-A. Bergovist, M. Hansson, et al. 1984. Chemistry and analysis of polychlorinated dioxins and dibenzofurans in biological samples. In: Biological Mechanisms of Dioxin Action. Banbury Report, A. Poland and R.D. Kimbrough, Ed. Cold Spring Harbor Laboratory. p. 17-25.

Rappe, C., M. Nygren, M. Hansson, et al. 1985. Identification of 2,3,7,8-substituted polychlorinated dioxins (PCDDs) and dibenzofurans (PCDFs) in environmental and human samples. (Submitted for publication)

Reggiani, G. 1980. Acute human exposure to TCDD in Seveso, Italy. J. Toxicol. Environ. Health. 6(1): 27-43.

Reggiani, G. 1982. Toxicology of TCDD and related compounds: Observation in man. In: Chlorinated Dioxins and Related Compounds. Impact on the Environment. O. Hutzinger, R.W. Frei, E. Merian and F. Pocchiari, Ed. Pergamon Press, NY. p. 463-493.

Riihimaki, V., S. Asp, A.M. Seppalainen and S. Herberg. 1978. Symptomatology, Morbidity, and Mortality Experience of Chlorinated Phenoxy Acid Herbicide (2,4-D and 2,4,5-T) Sprayers in Finland: A Clinical and Epidemiological Study. Working paper for an IARC working group meeting on coordination of epidemiological studies on the long-term hazards of chlorinated dibenzodioxins and chlorinated dibenzofurans. Lyon, France. Jan. 10-11.

Riihimaki, V., A. Sisko and S. Hernberg. 1982. Mortality of 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid herbicide applicators in Finland. Scand. J. Work Environ. Health. 8: 37-42.

Riihimaki, V., S. Asp, E. Pukkala and S. Hernberg. 1983. Mortality and cancer morbidity among chlorinated phenoxy acid applicators in Finland. Chemosphere. 12(4/5): 779-784.

Rikans, L.E., D.D. Gibson and P.B. McCay. 1979. Evidence for the presence of cytochrome P-450 in rat mammary gland. Biochem. Pharmacol. 28(19): 3039-3042.

Rizzardini, M., M. Romano, F. Turzi, et al. 1983. Toxicological evaluation of urban waste incinerator emissions. Chemosphere. 12: 559-564.

Roberts, E.A., N.H. Shear and A.B. Okey. 1985. The Ah receptor and dioxin toxicity: From rodent to human tissues. Chemosphere. 14(6/7): 661-674.

Robinson, J.R., J.S. Felton, R.C. Levitt, S.S. Thorgeirsson and D.W. Nebert. 1975. Relationship between aromatic hydrocarbon responsiveness and the survival times in mice treated with various drugs and environmental compounds. Mol. Pharmacol. 11: 850-865.

Rogers, A.M., M.E. Anderson and K.C. Back. 1982. Mutagenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin and perfluoro-n-decanoic acid in L5178y mouse lymphoma cells. Mutat. Res. 105: 445-449.

Roll, R. 1971. Studies of the teratogenic effect of 2,4,5-T in mice. Food Cosmet. Toxicol. 9(5): 671-676.

Rose, J.Q., J.C. Ramsey, T.H. Wentzler, R.A. Hummel and P.J. Gehring. 1976. The fate of 2,3,7,8-tetrachlorodibenzo-p-dioxin following single and repeated oral doses to the rat. Toxicol. Appl. Pharmacol. 36(2): 209-226.

Ryan, J.J. and J.C. Pilon. 1980. High performance liquid chromatography in the analysis of chlorinated dibenzodioxins and dibenzofurans in the chicken liver and wood shaving samples. J. Chromatogr. 197: 171-180.

Ryan, J.J., J.C. Pilon, H.B.S. Conacher and D. Firestone. 1983. Interlaboratory study on determination of 2,3,7,8-tetrachlorodibenzo-p-dioxin in fish. J. Assoc. Off. Anal. Chem. 66: 700-707.

Ryan, J.J., R. Lizotte and B.P-Y. Lau. 1985. Chlorinated dibenzo-p-dioxins and chlorinated dibenzofurnas in Canadian human adipose tissue. Chemosphere. 14(6/7): 697-706.

Safe, S. 1984. Polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs): Biochemistry, toxicology and mechanism of action. CRC Critical Reviews. Toxicology. 13: 319-343.

Safe, S. 1985. Personal communication to Dr. D. Mukerjee, ECAO, U.S. EPA, Cincinnati, OH.

Safe, S., L.W. Robertson, L. Safe, et al. 1982. Halogenated biphenyls: Molecular toxicology. Can. J. Physiol. Pharmacol. 60: 1057-1064.

Safe, S., T. Sawyer, S. Bandiera, et al. 1984. Binding to the 2,3,7,8-TCDD receptor and AHH/EROD induction: In vitro QXAR. In: Biological Mechanisms of Dioxin Action. Banbury Report 18. A. Poland and R.D. Kimbrough, Ed. Cold Spring Harbor Laboratory. p. 135-149.

SAI (Systems Applications, Inc.) 1980. Human Exposure to Atmospheric Concentration of Selected Chemicals. Vol. I. NTIS PB 81-193252.

Sarma, P.R. and J. Jacops. 1981. Thoracic soft-tissue sarcoma in Vietnam veterans exposed to agent orange. Lancet. 306(18): 1109.

Sawahata, T., J.R. Olson and R.A. Neal. 1982. Identification of metabolites 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) formed on incubation with isolated rat hepatocytes. Biochem. Biophys. Res. Commun. 105(1): 341-346.

Sawyer, T. and S. Safe. 1982. PCB isomers and congeners: Induction of aryl hydrocarbon hydroxylase and ethoxyresorufin O-deethylaes enzyme activities in rat hepatoma cells. Toxicol. Lett. 13: 87-94.

Sawyer, T., S. Bandiera, S. Safe, O. Hutzinger and K. Olie. 1983. Bioanalysis of polychlorinated dibenzofuran and dibenzo-p-dioxin mixtures in fly ash. Chemosphere. 12: 529-535.

Scarpelli, D.G., M.S. Rao, V. Subbarao, M. Beversluis, D.P. Gurka and P.F. Hollenberg. 1980. Activation of nitrosamines to mutagens by post-mitochondrial fraction of hamster pancreas. Cancer Res. 40(1): 67-74.

Schantz, S.L., D.A. Barsotti and J.R. Allen. 1979. Toxicological effects produced in nonhuman primates chronically exposed to fifty parts per trillion 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Toxicol. Appl. Pharmacol. 48: A180.

Schecter, A., J.J. Ryan, R. Lizotte, et al. 1985. Chlorinated dibenzodioxins and dibenzofurans in human adipose tissue from exposed and control New York State patients. Chemosphere. 14(6/7): 933-937.

Schueler, R.L. 1983. Review of Selected Neoplastic and Non-neoplastic Liver Lesions in Rats Given Hexachlorodibenzo-p-dioxins. Prepared for the U.S. EPA under Contract No. 68-02-3763.

Schwetz, B.A., J.M. Norris, G.L. Sparschu, et al. 1973. Toxicology of chlorinated dibenzo-p-dioxins. Environ. Health Perspect. 5: 87-99.

Seefeld, M.D. and R.E. Peterson. 1983. 2,3,7,8-Tetrachlorodibenzo-p-dioxin-induced weight loss: A proposed mechanism. _In_: Human and Environmental Risks of Chlorinated Dioxins and Related Compounds, R.E. Tucker, A.L. Young and A.P. Gray, Ed. Plenum Press, NY. p. 405-413.

Seefeld, M.D., R.M. Albrecht and R.E. Peterson. 1979. Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on indocyanine green blood clearance in rhesus monkeys. Toxicology. 14(3): 263-272.

Seefeld, M.D., R.M. Albrecht, K.W. Gilchrist and R.E. Peterson. 1980. Blood clearance tests for detecting 2,3,7,8-tetrachlorodibenzo-p-dioxin hepatotoxicity in rats and rabbits. Arch. Environ. Contam. Toxicol. 9(3): 317-327.

Seefeld, M.D., S.W. Corbett, R.E. Keesey and R.E. Peterson. 1984. Characterization of the wasting syndrome in rats treated with 2,3,7,8-tetrachlorodibenzo-_p_-dioxin. Toxicol. Appl. Pharmacol. 73: 311-322.

Seiler, J.P. 1973. A survey on the mutagenicity of various pesticides. Experientia. 29: 622-623.

Shadoff, L.A., R.A. Hummel, L. Lamparski and J.H. Davidson. 1977. A search for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in an environment exposed annually to 2,4,5-trichlorophenoxyacetic acid ester (2,4,5-T) herbicides. Bull. Environ. Contam. Toxicol. 18(4): 478-485.

Sharma, R.P. and P.J. Gehring. 1979. Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on splenic lymphocyte transformation in mice after single and repeated exposures. Ann. NY Acad. Sci. 320: 487-497.

Shaub, W.M. and W. Tsang. 1983. Physical and chemical properties of dioxins in relation to their disposal. In: Human and Environmental Risks of Chlorinated Dioxins and Related Compounds, R. Tucker et al., Ed. Plenum Publishing Corp., NY. p. 731-748.

Shiverick, K.T. and T.F. Muther. 1982. Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on serum concentrations and the uterotrophic action of exogenous estrone in rats. Toxicol. Appl. Pharmacol. 65(1): 170-176.

Shiverick, K.T. and T.F. Muther. 1983. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) effect on hepatic microsomal steroid metabolism and serum estradiol of pregnant rats. Biochem. Pharmacol. 32: 991-995.

Shum, S., N.M. Jensen and D.W. Nebert. 1979. The Ah Locus. In utero toxicity and teratogenesis associated with genetic differences in benzo[a]-pyrene metabolism. Teratology. 20: 365-376.

Silbergeld, E.K. 1984. Neurotoxicology of Agent Orange. Training course in neurotoxicology. Johns Hopkins School of Hygiene and Public Health. November.

Silkworth, J., D. McMartin, A. DeCaprio, R. Rej, P. O'Keefe and L. Kaminsky. 1982. Acute toxicity in guinea pigs and rabbits of soot from a polychlorinated biphenyl-containing transformer fire. Toxicol. Appl. Pharmacol. 65: 425-439.

Singer, R., M. Moses, J. Valciukas, R. Lilis and I.J. Selikoff. 1982. Nerve conduction velocity studies of workers employed in the manufacture of phenoxy herbicides. Environ. Res. 29: 297-311.

Sirchia, G. 1978. Report to Lombardy Regional Authority (unpublished). (Cited in Pocchiari et al., 1979)

Smith, A.H. 1983. School of Public Health, University of California. Letter to the Carcinogen Assessment Group, U.S. EPA, December 13.

Smith, A.H., J.E. Francis, S.J. Kay and J.B. Greig. 1981. Hepatic toxicity and uroporphyrinogen decarboxylase activity following a single dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin to mice. Biochem. Pharmacol. 30(20): 2825-2830.

Smith, A.H., D.O. Fisher, N. Pearce and C.J. Chapman. 1982a. Congenital defects and miscarriages among New Zealand 2,4,5-T sprayers. Arch. Environ. Health. 37: 197-200.

Smith, A.H., D.O. Fisher, N. Pearce and C.A. Teague. 1982b. Do agricultural chemicals cause soft-tissue sarcoma? Initial findings of a case-control study in New Zealand. Community Health Studies. 6(2): 114-119.

Smith, A.H., D.G. Fisher, H.J. Giles and N. Pearce. 1983a. The New Zealand soft tissue sarcoma case-control study: Interview findings concerning phenoxyacetic acid exposure. Chemosphere. 12(4/5): 565-571.

Smith, F.A., B.A. Schwetz and K.D. Nitschke. 1976. Teratogenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in CF-1 mice. Toxicol. Appl. Pharmacol. 38(3): 517-523.

Smith, J. 1979. EPA halts most uses of herbicide 2,4,5-T. Science. 203(4385): 1090-1091.

Smith, L.M., D.L. Stalling and J.L. Johnson. 1984. Determination of parts-per-trillion levels of polychlorinated dibenzofurans and dioxins in environmental samples. Anal. Chem. 56(11): 1830-1842.

Smith, R.M., P.W. O'Keefe, K.M. Aldous, D.R. Hilker and J.E. O'Brian. 1983b. 2,3,7,8-Tetrachlorodibenzo-p-dioxin in sediment and samples from Love Canal storm sewers and creeks. Environ. Sci. Technol. 17: 6-10.

Sparschu, G.L., Jr., F.L. Dunn, Jr., and V.K. Rowe, Jr. 1971a. Study of the teratogenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the rat. Food Cosmet. Toxicol. 9: 405-412.

Sparschu, G.L., F.L. Dunn, R.W. Lisowe and V.K. Rowe. 1971b. Effects of high levels of 2,4,5-trichlorophenoxyacetic acid on fetal development in the rat. Food Cosmet. Toxicol. 9(4): 527-530.

Spencer, H.W. and W. Woodrow. 1979. Memorandum to D. Reisa. TDAP review at the University of Wisconsin. TCDD in rats. February 8.

Squire, R.A. 1980. Pathologic Evaluations of Selected Tissues from the Dow Chemical TCDD and 2,4,5-T Rat Studies. Submitted to Carcinogen Assessment Group, U.S. EPA on August 15 under contract no. 68-01-5092.

Squire, R.A. 1983. An Assessment of the Experimental Evidence for Potential Carcinogenicity of Hexachlorodibenzo-p-dioxins. Report prepared for Vulcan Chemicals and Reichhold Chemicals, Inc. June 29, 1983.

SRI (Stanford Research Institute) International. 1982. 1982 Directory of Chemical Producers: USA, SRI International, Menlo Park, CA.

Stalling, D.L., L.M. Smith, J.D. Petty, et al. 1983. Residues of polychlorinated dibenzo-p-dioxins and dibenzofurans in Laurentian Great Lakes fish. In: Human and Environmental Risks of Chlorinated Dioxins and Related Compounds, R.E. Tucker et al., Ed. Plenum Press, NY. p. 220-240.

Stanley, J., C. Haile, A. Small and E. Olson. 1982. Sampling and Analysis Procedures for Assessing Organic Emissions from Stationary Combustion Sources for EED Studies. Methods-Manual. EPA 580/5-82-014. Office of Toxic Substances, U.S. EPA, Washington, DC.

Stehl, R.H., R.R. Paperfuss, R.A. Bredeweg and R.W. Roberts. 1973. The stability of pentachlorophenol and chlorinated dioxins to sunlight, heat and combustion. Adv. Chem. Ser. 120, Am. Chem. Soc. p. 119.

Stephan, C.E. 1980. Memorandum to Jerry F. Stara, U.S. EPA, Cincinnati, OH. July.

Stevens, K.M. 1981. Agent Orange toxicity: A quantitative perspective. Human Toxicol. 1(1): 31-39.

Stohs, S.J., M.Q. Hassan and W.J. Murray. 1983. Lipid peroxidation as a possible cause of TCDD toxicity. Biochem. Biophys. Res. Commun. 111: 854-859.

Strik, J.J. and J.M. de Wit. 1980. Health aspects of rabbits in a low-TCDD contaminated area. Int. J. Biochem. 12(5-6): 999-1001.

Sundstrom, G., S. Jensen, B. Jansson and K. Erne. 1979. Chlorinated phenoxyacetic acid derivatives and tetrachlorodibenzo-p-dioxin in foliage after application of 2,4,5-trichlorophenoxyacetic acid esters. Arch. Environ. Contam. Toxicol. 8(4): 441-448.

Suskind, R.R. and V.S. Hertzberg. 1984. Human health effects of 2,4,5-T and its toxic contaminants. J. Am. Med. Assoc. 251(18): 2372-2380.

Sweeney, G.D. and K.G. Jones. 1978. On the mechanism of porphyria due to chlorinated hydrocarbons. Int. Congr. Ser. Excerpta Med. 440: 229-231.

Sweeney, G.G. and K.G. Jones. 1983. Studies of the mechanism of action of hepatotoxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds. In: Human and Environmental Risks of Chlorinated Dioxins and Related Compounds, R.E. Tucker, A.L. Young and A.P. Gray, Ed. Plenum Press, NY. p. 415-422.

Sweeney, G.D., K.G. Jones, F.M. Cole, D. Basford and F. Krestynski. 1979. Iron deficiency prevents liver toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Science. 204(4390): 332-335.

Swift, L.L., T.A. Gasiewicz, G.D. Dunn, P.D. Soul'e and R.A. Neal. 1981. Characterization of the hyperlipidemia in guinea pigs induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol. Appl. Pharmacol. 59(3): 489-499.

Taylor, J.S. 1979. Environmental chloracne: Update and overview. Ann. NY Acad. Sci. 320: 295-307.

Taylor, M.L., T.O. Tiernan, J.H. Garrett, G.F. Van Ness and J.G. Solch. 1983. Assessments of incineration processes as sources of supertoxic chlorinated hydrocarbons: Concentrations of polychlorinated dibenzo-p-dioxins/dibenzofurans and possible precursor compounds in incinerator effluents. In: Chlorinated Dioxins and Dibenzofurans in the Total Environment, Vol. I, L.H. Keith et al., Ed. Butterworth Publishers, p. 125-164.

Teitelbaum, P.J. and A.P. Poland. 1978. Studies of the hepatic uptake of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the mouse. Fed. Proc. Fed. Am. Soc. Exp. Biol. 37(3): 692.

Tenchini, M.L., C. Crimaudo, G. Simoni, L. Decarli, R. Giorgi and F. Nuzzo. 1979. Approaches to the evaluation of genetic damage after a major hazard in chemical industry: Preliminary cytogenetic findings in TCDD-exposed subjects after the Seveso accident. In: Genetic Damage in Man Caused by Environmental Agents, K. Berg, Ed. Academic Press, NY. p. 301-316.

Tenchini, M.L., C. Crimaudo, G. Pacchetti, A. Motura, S. Agosti and L. DeCarli. 1983. A comparative cytogenetic study on cases of induced abortions in TCDD-exposed and nonexposed women. Environ. Mutagen. 5: 73-85.

Thibodeaux, L.J. 1979. Chemodynamics-Environmental Movement of Chemicals in Air, Water and Soil. John Wiley and Sons, Inc., NY. p. 176-177.

Thibodeaux, L. 1983. Off-site transport of 2,3,7,8-tetrachlorodibenzo-p-dioxin from a production disposal facility. In: Chlorinated Dioxins and Dibenzofurans in the Total Environment, Vol. I, L.H. Keith et al., Ed. Butterworth Publishers, Woburn, MA.

Thiess, A.M. and R. Frentzel-Beyme. 1977. Mortality Study of Persons Exposed to Dioxin Following an Accident which Occurred in the BASF on 13, November, 1953. Proceedings of MEDICHEM Congress V, San Francisco, Sept. 5.

Thiess, A.M. and R. Frentzel-Beyme. 1978. Mortality Study of Persons Exposed to Dioxin Following an Accident which Occurred in the BASF on 13, November, 1953. Working Papers, Joint NIEHS/IARC Working Group Report, Lyon, France, June, 1978.

Thiess, A.M., R. Frentzel-Beyme and R. Link. 1982. Mortality study of persons exposed to dioxin in a trichlorophenol-process accident that occurred in the BASF AG on November 17, 1953. Am. J. Ind. Med. 3: 179-189.

Thigpen, J.E., R.E. Faith, E.E. McConnell and J.A. Moore. 1975. Increased susceptibility to bacterial infection as a sequela of exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Infect. Immun. 12(6): 1319-1324.

Thomas, N.A. 1983. Memorandum to C.E. Stephan, U.S. EPA, Duluth, MN. July 22.

Thomas, P.E., J.J. Hutton and B.A. Taylor. 1973. Genetic relationship between aryl hydrocarbon hydroxylase inducibility and chemical carcinogen induced skin ulceration in mice. Genetics. 74: 655-659.

Thomas, P.T. and R.D. Hinsdill. 1979. The effect of perinatal exposure to tetrachlorodibenzo-p-dioxin on the immune response of young mice. Drug Chem. Toxicol. 2(1,2): 77-98.

Thomas, H.F. 1980a. Internal memo to P. Cohn, Office of Toxic Substances, U.S. EPA, Washington, DC.

Thomas, H.F. 1980b. 2,4,5-T use and congenital malformation rates in Hungary. Lancet. ii: 214-215.

Thunberg, T. 1984. Effects of TCDD on vitamin A and its relation to TCCD toxicity. In: Biological Mechanisms of Dioxin Action. Banbury Report 18. A. Poland and R.D. Kimbrough, Ed. Cold Spring Harbor Laboratory. p. 333-344.

Thunberg, T. and H. Håkansson. 1983. Vitamin A (retinol) status in the Gunn rat: The effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Arch. Toxicol. 53: 225-233.

Thunberg, T., U.G. Ahlborg and H. Johnsson. 1979. Vitamin A (retinol) status in the rat after a single oral dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Arch. Toxicol. 42(4): 265-274.

Thunberg, T., U.G. Ahlborg, H. Hakansson, C. Krantz and M. Monier. 1980. Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin on the hepatic storage of Retinol in rats with different dietary supplies of vitamin A (Retinol). Arch. Toxicol. (Berl). 45(4): 273-285.

Thunberg, T., U.G. Ahlborg and B. Wahlström. 1984. Comparison between the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin and six other compounds on the vitamin A storage, the UDP-glucuronosyltransferase and the aryl hydrocarbon hydroxylase activity in the rat liver. Arch. Toxicol. 55: 16-19.

Tiernan, T.O. 1982. Chlorodibenzodioxins and chlorodibenzofurans: An overview. In: Detoxication of Hazardous Wastes, J.H. Exner, Ed. Ann Arbor Science Publishers, Inc., Ann Arbor, MI. p. 243-260.

Tiernan, T.O. 1983. Analytical chemistry of polychlorinated dibenzo-p-dioxins and dibenzofurans: A review of the current status. In: Chlorinated Dioxins and Dibenzofurans in the Total Environment, Vol. I, L.H. Keith et al., Ed. Butterworth Publishers. p. 211-237.

Tiernan, T.O., M.L. Taylor, S.D. Erk, J.G. Solch and G. Van Ness. 1980. Dioxins. Vol. II. Analytical Method for Industrial Wastes. Final Report Industrial Environmental Research Lab., Cincinnati, OH. EPA 600/280-57. 80 p.

Tiernan, T.O., M.L. Taylor, J.G. Solch, G.F. Van Ness, J.H. Garrett and M.D. Porter. 1982a. Incineration of chemical wastes containing polychlorinated biphenyls: Assessment of tests conducted at Rollins Environmental Sciences, Deer Park, TX, and Energy Systems Co., El Dorado, AR. In: Detoxification of Hazardous Waste, J.H. Exner, Ed. Ann Arbor Science (Butterworth). p. 143-184.

Tiernan, T.O., M.L. Taylor, J.G. Solch, G.F. Van Ness and J.H. Garrett. 1982b. Characterization of toxic components in the effluent from a refuse-fired incinerator. Resour. Conserv. 9: 343-354.

Tofilon, P.J., P.G. Peters, R.P. Clement, D.M. Hardwicke and W.N. Piper. 1980. Depressed guinea pig testicular microsomal cytochrome P-450 content by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Life Sci. 27(10): 871-876.

Tognoni, G. and A. Bonaccorsi. 1982. Epidemiological problems with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Drug Metab. Rev. 13: 447-469.

Tosine, H. 1981. Method used by Ontario Ministry of the Environment primarily for the analysis of fish tissues and raw and untreated waters. In: Polychlorinated Dibenzo-p-Dioxins: Limitations to the Current Analytical Techniques, NRCC/NRC, Ottawa, Canada. p. 129-137.

Toth, K., J. Sugar, S. Somfai-Relle and J. Bence. 1978. Carcinogenic bioassay of the herbicide, 2,4,5-trichlorophenoxyethanol (TCPE) with different 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin) content in Swiss mice. Prog. Biochem. Pharmacol. 14: 82-93.

Toth, K., S. Somfai-Relle, J. Sugar and J. Bence. 1979. Carcinogenicity testing of herbicide 2,4,5-trichlorophenoxyethanol containing dioxin and of pure dioxin in Swiss mice. Nature (Lond). 278(5704): 548-549.

Townsend, J.C., K.M. Bodner, P.F. Van Peenen, R.D. Olsen and R.R. Cook. 1982. Survey of reproductive events of wives of employees exposed to chlorinated dioxins. Am. J. Epidemiol. 115(5): 695-713.

Tsushimoto, G., F. Matsumura and R. Sago. 1982. Fate of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in an outdoor pond and in model aquatic ecosystem. Environ. Toxicol. Chem. 1: 61-68.

Tukey, R.H., R.R. Hannah, M. Negishi, D.W. Nebert and H.J. Eisen. 1982. The Ah locus: Correlation of intranuclear appearance of inducer-receptor complex with induction of cytochrome $P_1$-450 mRNA. Cell. 31: 275-284.

Tulp, M.T.M. and O. Hutzinger. 1978. Rat metabolism of polychlorinated dibenzo-p-dioxins. Chemosphere. 9: 761-768.

Turner, J.N. and D.N. Collins. 1983. Liver morphology in guinea pigs administered either pyrolysis products of a polychlorinated biphenyl transformer fluid or 2,3,7,8-tetrachlorodibenzo-p-dioxins. Toxicol. Appl. Pharmacol. 67: 417-429.

United Kingdom Ministry of Agriculture, Fisheries and Food. 1983. Advisory committee on pesticides: Report on phenoxy acid herbicides. Whitehall Place, London, February 7.

U.S. EPA. 1978. Report of the ad hoc study broup on pentachlorophenol and contaminants. EPA/SAB/78/001. p. 170. (Cited in NRCC, 1981b)

U.S. EPA. 1979a. Chemistry Laboratory Manual for Bottom Sediments and Elutriate Testing. EPA 903/4-79-014. NTIS PB 294 596, Springfield, VA.

U.S. EPA. 1979b. Report of the FIFRA Scientific Advisory Panel on Proposed Section 6(b) (2) Notices for 2,4,5-T and Silvex. Memorandum from Dr. H. Wade Fowler, Jr., Executive Secretary FIFRA Scientific Advisory Panel to the Deputy Assistant Administrator for Pesticide Programs. Sept. 27, 1979.

U.S. EPA. 1979c. Report of Assessment of a Field Investigation of Six-year Spontaneous Abortion Rates in Three Oregon Areas in Relation to Forest 2,4,5-T Spray Practice. OTS, U.S. EPA.

U.S. EPA. 1980a. Dioxins. Industrial Pollution Control Division, IERL, ORD, U.S. EPA, Cincinnati, OH. EPA 600/2-80-197.

U.S. EPA. 1980b. Seafood Consumption Data Analysis, SRI International, Menlo Park, CA. Final Report, Task 11. EPA Contract No. 68-01-3887.

U.S. EPA. 1980c. Carcinogen Assessment Group's Risk Assessment on (2,4,5-Trichlorophenoxy)acetic acid (2,4,5-T), (2,4,5-Trichlorophenoxy)propionic acid (Silvex), and 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD). September 12.

U.S. EPA. 1982a. Test Method: 2,3,7,8-Tetrachlorodibenzo-p-dioxin-Method 613. Storet No. 34675. Environmental Monitoring and Support Laboratory, Cincinnati, OH.

U.S. EPA. 1982b. Environmental Monitoring at Love Canal. Vol. I. EPA 600/4-82-030a, Office of Research and Development, U.S. EPA, Washington, DC, May, 1982. NTIS PB 82-237330.

U.S. FPA. 1982c. Tolerances and Exemptions from Tolerances for Pesticide Chemicals in or on Raw Agricultural Commodities. Food Drug Cosmetic Law Reporter, 40 CFR 180.302.

U.S. EPA. 1983a. Dow Chemical Company -- Midland Plant Wastewater Characterization Study. Preliminary Summary of Results. March 28.

U.S. EPA. 1983b. Memorandum from U.S. EPA Cancer Assessment Group, to Judy Bellin, OSW, October 19.

U.S. EPA. 1984. Ambient Water Quality Criteria for 2,3,7,8-Tetrachlorodibenzo-p-dioxin. Environmental Criteria and Assessment Office, U.S. EPA, Cincinnati, OH. EPA 440/5-84-007.

USITC (U.S. International Trade Commission). 1982. Synthetic Organic Chemicals. United States Production and Sales, 1981. USITC Publ. No. 1292, Washington, DC.

Van der Berg, M., K. Olie and O. Hutzinger. 1983. Uptake and selective retention in rats of orally administered chlorinated dioxins and dibenzofurans from fly ash and fly ash extract. Chemosphere. 12(4/5): 537-544.

van Logten, M.J., B.N. Gupta, E.E. McConnell and J.A. Moore. 1980. Role of the endocrine system in the action of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on the thymus. Toxicology. 15(2): 135-144.

van Logten, M.J., B.N. Gupta, E.E. McConnell and J.A. Moore. 1981. The influence of malnutrition on the toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in rats. Toxicology. 21(1): 77-88.

Van Miller, J.P., R.J. Marlar and J.R. Allen. 1976. Tissue distribution and excretion of tritiated tetrachlorodibenzo-p-dioxin in non-human primates and rats. Food Cosmet. Toxicol. 14(1): 31-34.

Van Miller, J.P., J.J. Lalich and J.R. Allen. 1977a. Increased incidence of neoplasms in rats exposed to low levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Chemosphere. 6(10): 625-632.

Van Miller, J.P., J.J. Lalich and J.R. Allen. 1977b. Increased incidence of neoplasms in rats exposed to low levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Chemosphere. 6(9): 537-544.

Van Ness, G.F., J.G. Solch, M.L. Taylor and T.O. Tiernan. 1980. Tetrachlorodibenzo-p-dioxins in chemical wastes, aqueous effluents and soils. Chemosphere. 9: 553-563.

Vecchi, A., A. Mantovani, M. Sironi, W. Luini, F. Spreafico and S. Garattini. 1980. The effect of acute administration of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on humoral antibody production and cell-mediated activities in mice. Arch. Toxicol. (Suppl.) 4: 163-165.

Vecchi, A., M. Sironi, M.A. Canegrati, M. Recchia and S. Garattini. 1983. Immunosuppressive effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin in strains of mice with different susceptibility to induction of aryl hydrocarbon hydroxylase. Toxicol. Appl. Pharmacol. 68: 434-441.

Veith, G.D. and P. Kosian. 1983. Estimating bioconcentration potential from octanol/water partition coefficients. In: Physical Behavior of PCBs in the Great Lakes, D. Mackay et al., Ed. Ann Arbor Science, Ann Arbor, MI. p. 269.

Veith, G.D., K.J. Macek, S.R. Petrocelli and J. Carroll. 1980. An evaluation of using partition coefficients and water solubility to estimate bioconcentration factors for organic chemicals in fish. In: J.G. Eaton et al., Ed., Aquatic Toxicology. ASTM STP 707. American Society for Testing and Materials, Philadelphia, PA. p. 116-129.

Villaneuva, E.C., R.W. Jennings and V.W. Burse. 1973. Chlorodibenzo-p-dioxin contamination of two commercially available pentachlorophenols. J. Agric. Food Chem. 21(4): 739.

Vinopal, J.H. and J.E. Casida. 1973. Metabolic stability of 2,3,7,8-tetrachlorodibenzo-p-dioxin in mammalian liver microsomal systems and in living mice. Arch. Environ. Contam. Toxicol. 1(2): 122-132.

Vos, J.G. 1977. Immune suppression as related to toxicology. Crit. Rev. Toxicol. 5(1): 67-101.

Vos, J.G. and R.B. Beems. 1971. Dermal toxicity studies of technical polychlorinated biphenyls and fractions thereof in rabbits. Toxicol. Appl. Pharmacol. 19: 617-633.

Vos, J.G. and J.H. Koeman. 1970. Comparative toxicologic study with polychlorinated biphenyls in chickens with special reference to prophyrin, edema formation,- liver necrosis and tissue residues. Toxicol. Appl. Pharmacol. 17: 656-668.

Vos, J.G. and J.A. Moore. 1974. Suppression of cellular immunity in rats and mice by maternal treatment with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Int. Arch. Allergy Appl. Immunol. 47(5): 777-794.

Vos, J.G., J.A. Moore and J.G. Zinkl. 1973. Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin on the immune system of laboratory animals. Environ. Health Perspect. 5: 149-162.

Vos, J.G., J.A. Moore and J.G. Zinkl. 1974. Toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in C57Bl/6 mice. Toxicol. Appl. Pharmacol. 29: 229-241.

Vos, J.G., J.G. Kreeftenberg and L. Kater. 1978a. Immune Suppression by TCDD. In: Dioxin: Toxicological and Chemical Aspects. SD Medical and Scientific Books, NY. p. 163-175.

Vos, J.G., J.G. Kreeftenberg, H.W. Engel, A. Minderhoud and J.L.M. Van Noorle. 1978b. Studies on 2,3,7,8-tetrachlorodibenzo-p-dioxin induced immune suppression and decreased resistance to infection: Endotoxin hypersensitivity, serum zinc concentrations and effect of thymos in treatment. Toxicology. 9(1-2): 75-86.

Walker, A.E. and J.V. Martin. 1979. Lipid profiles in dioxin-exposed workers. Lancet. 1: 446-447.

Walsh, J. 1977. Seveso: The questions persist where dioxin created a wasteland. Science. 197(4308): 1064-1067.

Ward, A.M. 1982. Investigation of the immune capability of workers previously exposed to 2,3,7,8-tetrachlorodibenzo-para-dioxin (TCDD). In: The Chemical Scythe: Lessons of 2,4,5-T and Dioxin, A. Hay, Ed. Plenum Press, NY. p. 117-118.

Ward, C. and F. Matsumura. 1977. Fate of 2,4,5-T contaminant, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in aquatic environments. NTIS PB-264187. 28 p.

Ward, C.T. and F. Matsumura. 1978. Fate of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in a model aquatic environment. Arch. Environ. Contam. Toxicol. 7: 349-357.

Wassom, J.S., J.E. Huff and N. Loprieno. 1978. A review of the genetic toxicology of chlorinated dibenzo-p-dioxins. Mutat. Res. 47(3,4): 141-160.

Weber, G., P. Luzi, L. Resi, P. Tanganelli, M.R. Lovati and A. Poli. 1983. Natural history of TCDD-induced liver lesions in rats as observed by transmission electron microscopy during a 32-week period after a single intraperitoneal injection. J. Toxicol. Environ. Health. 12: 533-540.

Weber, H., H. Poiger and C. Schlatter. 1982. Fate of 2,3,7,8-tetrachlorodibenzo-p-dioxin metabolites from dogs in rats. Xenobiotica. 12(6): 353-357.

Weissberg, J.B. and J.G. Zinkl. 1973. Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin upon hemostasis and hematologic function in the rat. Environ. Health Perspect. 5: 119-123.

Whitlock, J.P., D.I. Israel, D.R. Galeazzi and A.G. Miller. 1984. 2,3,7,8-Tetrachlorodibenzo-p-dioxin regulates cytochrome $P_1$-450 gene expression. In: Biological Mechanisms of Dioxin Action. Banbury Report 18. A. Poland and R.D. Kimbrough, Ed. Cold Spring Harbor Laboratory. p. 191-200.

Wipf, H.K. and J. Schmid. 1983. Seveso -- An environmental assessment. In: Human and Environmental Risks of Chlorinated Dioxins and Related Compounds, R.E. Tucker et al., Ed. Plenum Publishing Corp., NY. p. 255-274.

Wipf, H.K., E. Homberger, N. Neimer, U.B. Ranalder, W. Vetter and J.P. Vuilleumeir. 1982. TCDD levels in soil and plant samples from the Seveso area. In: Chlorinated Dioxins and Related Compounds: Impact on the Environment, O. Hutzinger et al., Ed. Pergamon Press, NY. p. 115-126.

Wolfe, W.H., U.E. Michalek, R.A. Albanese, G.D. Lathrop and P.M. Moynahan. 1984. An epidemiologic investigation of health effects in Air Force personnel following exposure to herbicides. Mortality update, 1984. December 10. USAF School of Aerospace Medicine (AFSC), Brooks Air Force Base, TX.

Wolfe, W.H., G.D. Lathrop, R.A. Albanese and P.M. Moynahan. 1985. An epidemiologic investigation of health effects in Air Force personnel following exposure to herbicides and associated dioxins. Chemosphere. 14(6/7): 707-716.

Woods, J.S. 1973. Studies of the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on mammalian hepatic δ-aminolevulinic acid synthetase. Environ. Health Perspect. 5: 221.

Woolson, E.A., R.F. Thomas and P.D.J. Ensor. 1972. Survey of polychlorodibenzo-p-dioxin content in selected pesticides. 20(2): 350-354.

Yamagishi, T., T. Miyazaki, K. Akiyama, et al. 1981. Polychlorinated dibenzo-p-dioxins and dibenzofurans in commercial diphenyl ether herbicides, and in freshwater fish collected from the application area. Chemosphere. 10(10): 1137-1144.

Yamamoto, H., H. Yoshimura, M. Fujita and T. Yamamoto. 1976. Metabolic and toxicologic evaluation of 2,3,4,4',4'-pentachlorobiphenyl in rats and mice. Chem. Pharm. Bull. 24: 2168-2174.

Yang, K.H. and R.E. Peterson. 1977. Differential effects of halogenated aromatic hydrocarbons on pancreatic excretory function in rats. Fed. Proc. Fed. Am. Soc. Exp. Biol. 36(3): 356.

Yang, K.H., W.A. Croft and R.E. Peterson. 1977. Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxins on plasma disappearance and biliary excretion of foreign compounds in rats. Toxicol. Appl. Pharmacol. 40: 485-496.

Yang, K.H., E.J. Choi and S.Y. Choe. 1983. Cytotoxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin on primary cultures of adult rat hepatocytes. Arch. Environ. Contam. Toxicol. 12: 183-188.

Yockim, R.S., A.R. Isensee and G.E. Jones. 1978. Distribution and toxicity of TCDD and 2,4,5-T in an aquatic model ecosystem. Chemosphere. 7(3): 215-220.

Yoshihara, S., K. Nagata, H. Yoshimura, H. Kuroki and Y. Masuda. 1981. Inductive effect on hepatic enzymes and acute toxicity of individual polychlorinated dibenzofuran congeners in rats. Toxicol. Appl. Pharmacol. 59: 580-588.

Young, A.L. 1983. Long-term studies on the persistence and movement of TCDD in a natural ecosystem. In: Human and Environmental Risks of Chlorinated Dioxins and Related Compounds, R.E. Tucker et al., Ed. Plenum Publishing Corp., NY. p. 173-190.

Young, A.L., C.E. Thalken and W.E. Ward. 1975. Studies of the ecological impact of repetitive aerial applications of herbicides on the ecosystem of test area C-52A, Eglin AFB, Florida. Air Force Armament Laboratory, Eglin Air Force Base, Florida. Air Force Report No. AFATL-TR-75-142. 142 p. NTIS AD-A032773.

Young, A.L., C.E. Thalken, E.L. Arnold, J.M. Cupello and L.G. Cockerham. 1976. Fate of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in the environment: Summary and decontamination recommendations. Air Force Tech. Report No. USAFA-TR-76-18, U.S. Air Force Academy, CO. p. 41.

Young, A.L., H.K. Kang and B.M. Shepard. 1983. Chlorinated dioxins as herbicide contaminants. Environ. Sci. Technol. 17: 530A-540A.

Zack, J.A. and R.R. Suskind. 1980. The mortality experience of workers exposed to tetrachlorodibenzodioxin in a trichlorophenol process accident. J. Occup. Med. 22(1): 11-14.

Zimmering, S., J.M. Mason, R. Valencia and R.C. Woodruff. 1985. Chemical mutagenesis testing in Drosophila. II. Results of 20 coded compounds tested for the National Toxicology Program. Environ. Mutagen. 7: 87-100.

Zimmermann, E.F. and D. Bowen. 1972. Distribution and metabolism of triamcinolone acetonide in inbred mice with different cleft palate sensitivities. Teratology. 5: 335-344.

Zinkl, J.G., J.G. Vos, J.A. Moore and B.N. Gupta. 1973. Hematologic and clinical chemistry effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin in laboratory animals. Environ. Health Perspect. 5: 111-118.

Zullei, N. and G. Benecke. 1978. Application of a new bioassay to screen the toxicity of polychlorinated biphenyls on blue-green algae. 20(6): 786-792.

# APPENDIX A

## TABLE A-1

## Cumulative Mortality of Male Rats[a]

|  |  | μg/kg/day 2,3,7,8-TCDD |  |  |
|---|---|---|---|---|
| Time (end of 30-day period) N= | Controls (86) | 0.1 (50) | 0.01 (50) | 0.001 (50) |
| 1-7 | 0.0 | 0.0 | 0.0 | 2.0 |
| 8 | 0.0 | 2.0 | 0.0 | 2.0 |
| 9 | 0.0 | 4.0 | 0.0 | 2.0 |
| 10 | 0.0 | 4.0 | 0.0 | 2.0 |
| 11 | 2.3 | 4.0 | 0.0 | 2.0 |
| 12 | 5.8 | 8.0 | 0.0 | 2.0 |
| 13 | 7.0 | 12.0 | 0.0 | 2.0 |
| 14 | 10.5 | 18.0 | 4.0 | 4.0 |
| 15 | 12.8 | 18.0 | 14.0 | 14.0 |
| 16 | 16.3 | 20.0 | 22.0 | 14.0 |
| 17 | 18.6 | 28.0 | 28.0 | 24.0 |
| 18 | 24.4 | 34.0 | 34.0 | 44.0[b] |
| 19 | 31.4 | 44.0 | 46.0 | 50.0 |
| 20 | 41.9 | 46.0 | 54.0 | 56.0 |
| 21 | 48.8 | 62.0 | 68.0 | 60.0 |
| 22 | 58.1 | 74.0[b] | 76.0[b] | 68.0 |
| 23 | 69.8 | 78.0 | 84.0 | 74.0 |
| 24 | 77.9 | 84.0 | 88.0 | 76.0 |
| 25 | 82.6 | 90.0 | 92.0 | 78.0 |

[a] Source: Kociba et al., 1977

[b] Interval of greatest difference, D, in cumulative mortality curves of controls and treatment group. None of the differences were statistically significant (Kolmogorov-Smirnov test, $p>0.05$).

## TABLE A-2

### Cumulative Mortality of Female Rats[a]

| Time<br>(end of 30-day period)<br>N= | Controls<br>(86) | µg/kg/day 2,3,7,8-TCDD<br>0.1<br>(50) | 0.01<br>(50) | 0.001<br>(50) |
|---|---|---|---|---|
| 0-5 | 0.0 | 0.0 | 0.0 | 0.0 |
| 6-8 | 1.2 | 0.0 | 0.0 | 0.0 |
| 9 | 1.2 | 2.0 | 0.0 | 0.0 |
| 10 | 1.2 | 4.0 | 2.0 | 0.0 |
| 11 | 1.2 | 8.0 | 2.0 | 0.0 |
| 12 | 1.2 | 16.0 | 4.0 | 4.0 |
| 13 | 3.5 | 20.0 | 4.0 | 4.0 |
| 14 | 3.5 | 26.0 | 8.0 | 6.0 |
| 15 | 7.0 | 28.0 | 12.0 | 10.0 |
| 16 | 12.8 | 32.0 | 18.0 | 12.0 |
| 17 | 15.1 | 38.0 | 18.0 | 18.0 |
| 18 | 18.6 | 44.0 | 20.0 | 22.0 |
| 19 | 25.6 | 56.0[b] | 30.0 | 34.0[b] |
| 20 | 34.9 | 60.0 | 36.0 | 36.0 |
| 21 | 40.7 | 66.0 | 46.0[b] | 44.0 |
| 22 | 58.1 | 82.0 | 60.0 | 52.0 |
| 23 | 64.0 | 86.0 | 66.0 | 58.0 |
| 24 | 70.9 | 88.0 | 72.0 | 66.0 |
| 25 | 70.9 | 92.0 | 72.0 | 68.0 |

[a] Source: Kociba et al., 1977

[b] Interval of greatest difference, D, in cumulative mortality curves of controls and treatment group. The mortality curve for the rats fed 0.1 µg/kg/day differed significantly from that for controls (D = 30.4, $p<0.01$, Kolmogorov-Smirov test). The other two groups did not differ significantly from controls ($p>0.05$).

## TABLE A-3

### Males: Interval Mortality Rates

| Days | Control | | 0.1 µg/kg/day | | 0.01 µg/kg/day | | 0.001 µg/kg/day | |
|---|---|---|---|---|---|---|---|---|
| | d/l | Rate | d/l | Rate | d/l | Rate | d/l | Rate |
| 40-30 | 0/86 | 0.000 | 0/50 | 0.000 | 0/50 | 0.000 | 1/50 | 0.020 |
| 31-210 | 0/86 | 0.000 | 0/50 | 0.000 | 0/50 | 0.000 | 0/49 | 0.000 |
| 211-240 | 0/86 | 0.000 | 1/50 | 0.020 | 0/50 | 0.000 | 0/49 | 0.000 |
| 241-270 | 0/86 | 0.000 | 1/49 | 0.020 | 0/50 | 0.000 | 0/49 | 0.000 |
| 271-300 | 0/86 | 0.000 | 0/48 | 0.000 | 0/50 | 0.000 | 0/49 | 0.000 |
| 301-330 | 2/86 | 0.023 | 0/48 | 0.000 | 0/50 | 0.000 | 0/49 | 0.000 |
| 331-360 | 3/84 | 0.036 | 2/48 | 0.042 | 0/50 | 0.000 | 0/49 | 0.000 |
| 391-420 | 3/80 | 0.038 | 3/44 | 0.068 | 2/50 | 0.040 | 1/49 | 0.020 |
| 421-450 | 2/77 | 0.026 | 0/41 | 0.000 | 5/48 | 0.104 | 5/48 | 0.104 |
| 451-480 | 3/75 | 0.040 | 1/41 | 0.024 | 4/43 | 0.093 | 0/43 | 0.000 |
| 481-510 | 2/72 | 0.028 | 4/40 | 0.100 | 3/39 | 0.077 | 5/43 | 0.116 |
| 511-540 | 5/70 | 0.071 | 3/36 | 0.083 | 3/36 | 0.083 | 10/38 | 0.263 |
| 541-570 | 6/65 | 0.092 | 5/33 | 0.152 | 6/33 | 0.182 | 3/28 | 0.107 |
| 571-600 | 9/59 | 0.153 | 1/28 | 0.036 | 4/27 | 0.148 | 3/25 | 0.120 |
| 601-630 | 6/50 | 0.120 | 8/27 | 0.296 | 7/23 | 0.304 | 2/22 | 0.091 |
| 631-660 | 8/44 | 0.182 | 6/19 | 0.316 | 4/16 | 0.250 | 4/20 | 0.200 |
| 661-690 | 10/36 | 0.278 | 2/13 | 0.154 | 4/12 | 0.333 | 3/16 | 0.188 |
| 691-720 | 7/26 | 0.269 | 3/11 | 0.273 | 2/8 | 0.250 | 1/13 | 0.077 |
| 721-726 | 4/19 | 0.211 | 3/8 | 0.375 | 2/6 | 0.333 | 1/12 | 0.083 |
| Terminal Kill | 15 | | 5 | | 4 | | 11 | |

Corrected for continuity for combined interval:

421-510 7/77 vs. 5/41($X^2$=0.04, n.s.) 12/48($X^2$= 4.63, $p<0.05$)
10/48 ($X^2$=2.54, n.s.

451-540 10/72 vs. 8/41($X^2$=0.37, n.s.) 10/43($X^2$=1.27, n.s.)
15/43 ($X^2$=6.37, $p<0.025$)

481-570 13/72 vs. 12/40($X^2$=1.48, n.s.) 12/39($X^2$=1.67, n.s.)
18/43 ($X^2$=6.59, $p<0.025$)

511-600 20/70 vs. 9/36($X^2$=0.03, n.s.) 13/36($X^2$=0.32, n.s.)
16/38 ($X^2$= 1/47, n.s.)

## TABLE A-4

## Females: Interval Mortality Rates

| Days | Control | | 0.1 μg/kg/day | | 0.01 μg/kg/day | | 0.001 μg/kg/day | |
|---|---|---|---|---|---|---|---|---|
| | d/l | Rate | d/l | Rate | d/l | Rate | d/l | Rate |
| 0-150 | 0/86 | 0.000 | 0/50 | 0.000 | 0/50 | 0.000 | 0/50 | 0.000 |
| 151-180 | 1/86 | 0.012 | 0/50 | 0.000 | 0/50 | 0.000 | 0/50 | 0.000 |
| 181-240 | 0/85 | 0.000 | 0/50 | 0.000 | 0/50 | 0.000 | 0/50 | 0.000 |
| 241-270 | 0/85 | 0.000 | 1/50 | 0.020 | 0/50 | 0.000 | 0/50 | 0.000 |
| 271-300 | 0/85 | 0.000 | 1/49 | 0.020 | 1/50 | 0.020 | 0/50 | 0.000 |
| 301-330 | 0/85 | 0.000 | 2/48 | 0.042 | 0/49 | 0.000 | 0/50 | 0.000 |
| 331-360 | 0/85 | 0.000 | 4/46 | 0.087 | 1/49 | 0.020 | 2/50 | 0.040 |
| 361-390 | 2/85 | 0.024 | 2/42 | 0.048 | 0/48 | 0.000 | 0/48 | 0.000 |
| 391-420 | 0/83 | 0.000 | 3/40 | 0.075 | 2/48 | 0.042 | 1/48 | 0.021 |
| 421-450 | 3/83 | 0.036 | 1/37 | 0.027 | 2/46 | 0.044 | 2/47 | 0.043 |
| 451-480 | 5/80 | 0.063 | 2/36 | 0.056 | 3/44 | 0.068 | 1/45 | 0.022 |
| 481-510 | 2/75 | 0.027 | 3/34 | 0.088 | 0/41 | 0.000 | 3/44 | 0.068 |
| 511-540 | 3/73 | 0.041 | 3/31 | 0.097 | 1/41 | 0.024 | 2/41 | 0.049 |
| 541-570 | 6/70 | 0.086 | 6/28 | 0.214 | 5/40 | 0.125 | 6/39 | 0.154 |
| 571-600 | 8/64 | 0.125 | 2/22 | 0.091 | 3/35 | 0.086 | 1/33 | 0.030 |
| 601-630 | 5/56 | 0.089 | 3/20 | 0.150 | 5/32 | 0.156 | 4/32 | 0.125 |
| 631-660 | 15/51 | 0.294 | 8/17 | 0.471 | 7/27 | 0.259 | 4/28 | 0.143 |
| 661-690 | 5/36 | 0.139 | 2/9 | 0.222 | 3/20 | 0.150 | 3/24 | 0.125 |
| 691-720 | 6/31 | 0.194 | 1/7 | 0.143 | 3/17 | 0.177 | 4/21 | 0.191 |
| 721-726 | 0/25 | 0.000 | 2/6 | 0.333 | 0/14 | 0.000 | 1/17 | 0.059 |
| Terminal Kill | 25 | | 4 | | 14 | | 16 | |

Corrected for continuity for combined interval:

421-510 10/83 vs. 6/37($X^2$=1.131 n.s.) 5/46($X^2$=0.0, n.s.)
6/47 ($X^2$=0.01, n.s.)

451-540 10/80 vs. 8/36($X^2$=1.13, n.s.) 4/44($X^2$=0.8, n.s.)
6/45 ($X^2$=0.01, n.s.)

481-570 11/75 vs. 12/34($X^2$=4.80, $p<0.05$) 6/41($X^2$=0.0, n.s.)
11/44 ($X^2$=1.34, n.s.)

510-600 17/73 vs. 11/31($X^2$=1.08, n.s.) 9/41($X^2$=0.0, n.s.)
9/41 ($X^2$=0.0, n.s.)

# APPENDIX B

## Tables for 2,3,7,8-TCDD Quantitative Incremental Unit Cancer Risk Estimates

## Tables for 2,3,7,8-TCDD Quantitative Incremental Unit Cancer Risk Estimates

Tables B-1 through B-5 are the 2,3,7,8-TCDD bioassay results judged suitable for quantitative estimates of incremental unit risk. Tables B-1 and B-2 show the results of the Dow rat feeding study for both males and females. The results include both the original (Kociba) analysis and the (Squire) review. Individual organ sites where significantly increased tumors occurred are tabulated separately, then the total number of animals with at least one of these tumors is compiled. Tables B-3, B-4 and B-5 compile similar data for the NCI bioassay. Table B-6 uses the data from Table B-1 to estimate the parameters of the linearized multistage model. The $X^2$ test for goodness-of-fit of the model to the data determines whether or not the highest dose group is retained in the fit. The 95% upper-limit on the linear term ${}_aq_1{}^*$ is then adjusted by the surface area constant $(70/W_a)^{1/3}$ to derive the final extrapolated animal-to-human 95% upper-limit incremental unit cancer risk estimate. Tables B-7 through B-12 present the extrapolation procedure for the remaining data sets, with Tables B-8A and B-9A adjusting for high early mortality in the female rat high-dose group. Table B-13 summarizes the estimates derived in Tables B-6 through B-12. The $q_1{}^*$ estimates from the female rat data of the Dow feeding study using both the Kociba and Squire readings are averaged to derive the final estimate $q_1{}^* = 1.56\text{x}10^5$ (mg/kg/day)$^5$.

### Description of the Animal-to-Human Extrapolation Procedure Using the Linearized Multistage Model

Let P(d) represent the lifetime risk (probability) of cancer at dose d. The multistage model has the form

$$P(d) = 1 - \exp\left[-(q_0 + q_1d + q_2d^2 + \ldots + q_kd^k)\right]$$

where

$$q_i \geq 0, \; i = 0, 1, 2, \ldots, k$$

Equivalently,

$$P_t(d) = 1 - \exp\left[(q_1 d + q_2 d^2 + \ldots + q_k d^k)\right]$$

where

$$P_t(d) = \frac{P(d) - P(0)}{1 - P(0)}$$

is the extra risk over background rate at dose d.

The point estimate of the coefficients $q_i$, $i = 0, 1, 2, \ldots, k$. and consequently, the extra risk function, $P_t(d)$, at any given dose d, is calculated by maximizing the likelihood function of the data.

The point estimate and the 95% upper confidence limit of the extra risk, $P_t(d)$, are calculated by using the computer program GLOBAL 79 developed by Crump and Watson (1979). At low doses, upper 95% confidence limits on the extra risk and lower 95% confidence limits on the dose producing a given risk are determined from a 95% upper confidence limit, $q_1^*$ on parameter $q_1$. Whenever $q_1 > 0$, at low doses the extra risk $P_t(d)$ has approximately the form $P_t(d) = q_1 \times d$. Therefore, $q_1^* \times d$ is a 95% upper confidence limit on the extra risk and $P_t/q_1^*$ is a 95% lower confidence limit on the dose producing an extra risk of $P_t$. Let $L_0$ be the maximum value of the log-likelihood function. The upper limit, $q_1^*$, is calculated by increasing $q_1$ to a value $q_1^*$ such that when the log-likelihood is remaximized subject to this fixed value $q_1^*$ for the linear coefficient, the resulting maximum value of the log-likelihood $L_1$ satisfies the equation

$$2\,(L_0 - L_1) = 2.70554$$

where 2.70554 is the cumulative 90% point of the chi-square distribution with one degree of freedom, which corresponds to a 95% upper limit (one-sided). This approach of computing the upper confidence limit for the extra risk, $P_t(d)$, is an improvement on the Crump et al. (1977) model. The upper confidence limit for the extra risk calculated at low doses is always linear. This is conceptually consistent with the linear nonthreshold. The slope, $q_1^*$, is taken as a plausible upper bound of the potency of the chemical in inducing cancer at low doses. (In the section calculating the risk estimates, $P_t(d)$ is abbreviated as P.)

In fitting the dose-response model, the number of terms in the polynomial is chosen equal to (h-1), were h is the number of dose groups in the experiment, including the control group.

Whenever the multistage model does not fit the data sufficiently well, data at the highest dose are deleted and the model is refit to the rest of the data. This is continued until an acceptable fit to the data is obtained. To determine whether or not a fit is acceptable, the chi-square

$$X^2 = \sum_{i=1}^{h} \frac{(R_i - N_iP_i)^2}{N_iP_i(1-P_i)}$$

statistic is calculated where $N_i$ is the number of animals in the $i^{th}$ dose group, $R_i$ is the number of animals in the $i^{th}$ dose group with a tumor response, $P_i$ is the probability of a response in the $i^{th}$ dose group estimated by fitting the multistage model to the data, and h is the number of remaining groups. The fit is determined to be unacceptable whenever $X^2$ is larger than the cumulative 99% point of the chi-square distribution with f degrees of freedom, where f equals the number of dose groups minus the number of nonzero multistage coefficients.

## TABLE B-1

DOW (Dr. Kociba) 2,3,7,8-TCDD Oral Rat Study (1978) with Dr. R. Squire's Review
Male Sprague-Dawley Rats - Spartan Substrain (2 yrs)*

| Tissue and Diagnosis | Dose Levels (μg/kg/day) | | | |
|---|---|---|---|---|
| | 0 (control) | 0.001 | 0.01 | 0.1 |
| Dow (Kociba) Analysis | | | | |
| 1. Tongue<br>Stratified squamous cell carcinoma | 0/76 (0%) | 1/49 (2%) | 1/49 (2%) | 3/42 (7%)<br>(p=0.043) |
| 2. Nasal turbinates/hard palate<br>Squamous cell carcinoma | 0/51 (0%) | 1/34 (3%) | 0/27 (0%) | 4/30 (13%)<br>(p=0.016) |
| Total | 0/76 (0%) | 2/49 (4%) | 1/49 (4%) | 7/42 (17%)<br>($p=5.12\times10^{-4}$) |
| R. Squire's Review | | | | |
| 1. Tongue<br>Squamous cell carcinoma | 0/77 (0%) | 1/44 (2%) | 1/49 (2%) | 3/44 (7%)<br>($p=4.60\times10^{-2}$) |
| 2. Nasal turbinates/hard palate<br>Squamous cell carcinoma | 0/55 (0%) | 1/34 (3%) | 0/26 (0%) | 6/30 (20%)<br>($p=1.36\times10^{-3}$) |
| Total (1 or 2 above)<br>(each rat had at least one tumor above) | 0/77 (0%) | 2/44 (5%) | 1/49 (2%) | 9/44 (20%)<br>($p=6.28\times10^{-5}$) |

*Average body weight of male rat = 600 g

## TABLE B-2

DOW (Dr. Kociba) 2,3,7,8-TCDD Oral Rat Study (1978) with Dr. R. Squire's Review
Female Sprague-Dawley Rats - Spartan Substrain (2 yrs)*

| Tissue and Diagnosis | Dose Levels (μg/kg/day) | | | |
|---|---|---|---|---|
| | 0 (control) | 0.001 | 0.01 | 0.1 |
| Dow (Kociba) Analysis | | | | |
| 1. Lung<br>Keratinizing squamous cell carcinoma | 0/86 (0%) | 0/50 (0%) | 0/49 (0%) | 7/49 (14%)<br>($p=6.21\times10^{-4}$) |
| 2. Nasal turbinates/hard palate<br>Stratified squamous cell carcinoma (revised diagnoses 2/19/79) | 1/54 (2%) | 0/30 (0%) | 1/27 (4%) | 5/24 (21%)<br>($p=9.46\times10^{-3}$) |
| 3. Liver<br>Hepatocellular hyperplastic nodules/hepatocellular carcinoma | 9/86 (10%) | 3/50 (6%) | 18/50 (36%)<br>(2 had both)<br>($p=4.37\times10^{-4}$) | 34/48 (71%)<br>($p=9.53\times10^{-13}$) |
| Total (1, 2, or 3 above) (each rat had at least one tumor above) | 9/86 (10%) | 3/50 (6%) | 18/50 (36%)<br>($p=4.37\times10^{-4}$) | 34/49 (69%)<br>($p=2.13\times10^{-12}$) |

TABLE B-2 (cont.)

| Tissue and Diagnosis | Dose Levels (μg/kg/day) | | | |
|---|---|---|---|---|
| | 0 (control) | 0.001 | 0.01 | 0.1 |
| R. Squire's Review | | | | |
| 1. Lung<br>Squamous cell carcinoma | 0/86 (0%) | 0/50 (0%) | 0/49 (0%) | 8/47 (17%)<br>($p=1.61 \times 10^{-4}$) |
| 2. Nasal turbinate/hard palate<br>Squamous cell carcinoma | 0/54 (0%) | 0/30 (0%) | 1/27 (4%) | 5/22 (23%)<br>($p=1.43 \times 10^{-3}$) |
| 3. Liver<br>Neoplastic nodules/hepatocellular carcinoma | 16/86 (0%) | 8/50 (16%) | 27/50 (54%)<br>($p=2.42 \times 10^{-5}$) | 33/47 (70%)<br>($p=4.92 \times 10^{-9}$) |
| Total combined (1, 2 or 3 above) (each animal had at least one tumor above) | 16/86 (19%) | 8/50 (16%) | 27/50 (54%)<br>($p=2.42 \times 10^{-5}$) | 34/47 (72%)<br>($p=1.20 \times 10^{-9}$) |

*Average body weight of female rat = 450 g

## TABLE B-3

### NCI 2,3,7,8-TCDD (Gavage) Bioassay (No. 80-1765)
### Osborne-Mendel Female Rats (2 years; weight = 450 g)

| Tissue and Diagnosis | Dose Levels (μg/kg/week) | | | |
|---|---|---|---|---|
| | Vehicle Control<br>0 | Low<br>0.01 | Medium<br>0.05 | High<br>0.5 |
| 1. Liver<br>Neoplastic nodule or hepatocellular carcinoma | 5/75 (7%) | 1/49 (2%) | 3/50 (6%) | 14/49 (28%)<br>(p=0.001) |
| 2. Adrenal*<br>Cortical adenoma, or carcinoma | 11/73 (15%) | 9/49 (18%) | 5/49 (10%) | 14/46 (30%)<br>(p=0.038) |

*The biological significance of this tumor in old rats is questionable, since it is commonly observed in control rats and associated with the aging process.

## TABLE B-4

NCI 2,3,7,8-TCDD (Gavage) Bioassay (No. 80-1765)
B6C3F1 Male Mice (2 years; weight = 48 g)

| | Dose Levels (µg/kg/week) | | | |
|---|---|---|---|---|
| Tissue and Diagnosis | Vehicle Control<br>0 | Low<br>0.01 | Medium<br>0.05 | High<br>0.5 |
| Liver | | | | |
| Hepatocellular adenoma or carcinoma | 15/73 (21%)<br>(p<0.001)[a] | 12/49 (24%) | 13/49 (26%) | 27/50 (54%)<br>($p=1.31\times10^{-4}$) |
| Hepatocellular carcinoma[b] | 8/73 (11%)<br>(p<0.001)[a] | 9/49 (18%) | 8/49 (16%) | 17/50 (34%)<br>(p=0.002) |

[a] Cochran-Armitage test for linear trend

[b] Used for Unit Risk Estimate

## TABLE B-5

## NCI 2,3,7,8-TCDD (Gavage) Bioassay (No. 80-1765)
## B6C3F1 Female Mice (2 years)[a]

| Tissue and Diagnosis | Dose Levels (μg/kg/week)<br>Vehicle Control<br>0 | Low<br>0.04 | Medium<br>0.2 | High<br>2.0 |
|---|---|---|---|---|
| 1. Subcutaneous tissue<br>Fibrosarcoma | 1/74 (1%) | 1/50 (2%) | 1/48 (2%) | 5/47 (11%)<br>(p=0.032) |
| 2. Hematopoietic system<br>Lymphoma or leukemia | 18/74 (24%) | 12/50 (24%) | 13/48 (27%) | 20/47 (43%)<br>(p=0.028) |
| 3. Liver<br>Hepatocellular adenoma or carcinoma | 3/73 (4%)<br>(p=0.0050) | 6/50 (12%) | 6/48 (12%) | 11/47 (23%)<br>($p=1.84\times10^{-3}$) |
| Hepatocellular carcinoma | 1/73 (1%)<br>(p=0.008)[b] | 2/50 (4%) | 2/48 (4%) | 6/47 (13%)<br>(p=0.014) |
| 4. Thyroid<br>Follicular cell adenoma | 0/69 | 3/50 (6%) | 1/47 (2%) | 5/46 (11%)<br>($p=8.93\times10^{-3}$) |
| Total (1, 2, 3 or 4 above)<br>(each mouse had at least one tumor above) | 22/74 (30%) | 20/50 (40%) | 19/48 (40%) | 31/47 (66%)<br>($p=8.94\times10^{-5}$) |

[a] Average body weight of female mouse = 40 g

[b] Cochran-Armitage test for trend

## TABLE B-6

### Curve Fit of the Multistage Model Parameters to Experimental Data by Study and Pathologist Linear Parameter $q_1$, Maximized to Give Upper 95% Limit $q_1^*$

Compound......................2,3,7,8-TCDD
Study.........................Kociba - Dow
Sex-species...................Male rat
Weight ($w_a$)..................600 g
Tumor sites (one or more)....Tongue - squamous cell carcinomas
Nasal turbinates/hard palate - stratified squamous cell carcinoma
(ref. Table B-1)

Pathologist - Kociba

| Exposure level (mg/kg/day) | 0 | $1 \times 10^{-6}$ | $1 \times 10^{-5}$ | $1 \times 10^{-4}$ |
|---|---|---|---|---|
| +r/n | 0/76 | 2/49 | 1/49 | 7/42 |

+r = number of animals with one or more of the tumors
n = total number of animals examined

| Estimated multistage parameters | $q_0$ | $q_1$ | $q_2$ | $q_3$ | $_aq_1^*$ | Goodness of fit $X^2$ |
|---|---|---|---|---|---|---|
| When all dose groups are used | $1.40 \times 10^{-2}$ | $1.10 \times 10^{3}$ | 0 | $5.86 \times 10^{10}$ | $3.01 \times 10^{3}$ | 3.34 (d.f. = 2) |
| When the highest dose group is not used | Above fit is satisfactory | | | | | |

$_aq_1^*$ = the maximum linear component from the model with adequate goodness of fit ($p>0.01$) = $3.01\times10^{3}$ $(\text{mg/kg/day})^{-1}$

$q_1^*$ = $_aq_1^* \ (70/w_a)^{1/3} = 1.47\times10^{4} \ (\text{mg/kg/day})^{-1}$, the upper 95% limit slope factor associated with human dose response.

## TABLE B-7

### Curve Fit of the Multistage Model Parameters to Experimental Data by Study and Pathologist Linear Parameter $q_1$, Maximized to Give Upper 95% Limit $q_1^*$

Compound......................2,3,7,8-TCDD
Study.........................Dow
Sex-species...................Male rat
Weight ($w_a$)..................600 g
Tumor sites (one or more)....Nasal turbinates/hard palate - squamous cell carcinoma
Tongue - squamous cell carcinoma (ref. Table B-1)

Pathologist - Squire

| Exposure level (mg/kg/day) | 0 | $1 \times 10^{-6}$ | $1 \times 10^{-5}$ | $1 \times 10^{-4}$ |
|---|---|---|---|---|
| +r/n | 0/77 | 2/44 | 1/49 | 9/44 |

+r = number of animals with one or more of the tumors
n = total number of animals examined

| Estimated multistage parameters | $q_0$ | $q_1$ | $q_2$ | $q_3$ | $aq_1^*$ | Goodness of fit $X^2$ |
|---|---|---|---|---|---|---|
| When all dose groups are used | 0.015 | $1.05 \times 10^3$ | 0 | $109.40 \times 10^9$ | $3.53 \times 10^3$ | 3.90 (d.f. = 1) |
| When the highest dose group is not used | | | | Above fit is satisfactory | | |

$aq_1^*$ = the maximum linear component from the model with adequate goodness of fit ($p>0.01$) = $3.53 \times 10^3$ $(mg/kg/day)^{-1}$

$q_1^*$ = $aq_1^*$ $(70/w_a)^{1/3}$ = $1.73 \times 10^4$ $(mg/kg/day)^{-1}$, the upper 95% limit slope factor associated with human dose response.

## TABLE B-8

### Curve Fit of the Multistage Model Parameters to Experimental Data by Study and Pathologist Linear Parameter $q_1$, Maximized to Give Upper 95% Limit $q_1^*$

Compound.....................2,3,7,8-TCDD
Study........................Dow
Sex-species..................Female rat
Weight ($w_a$)...................450 g
Tumor sites (one or more)....Liver, lung, hard palate, or nasal turbinates (ref. Table B-2)

Pathologist - Kociba

| Exposure level (mg/kg/day) | 0 | 1 x $10^{-6}$ | 1 x $10^{-5}$ | 1 x $10^{-4}$ |
|---|---|---|---|---|
| +r/n | 9/86 | 3/50 | 18/50 | 34/49 |

+r = number of animals with one or more of the tumors
n = total number of animals examined

| Estimated multistage parameters | $q_0$ | $q_1$ | $q_2$ | $q_3$ | $_aq_1^*$ | Goodness of fit $X^2$ |
|---|---|---|---|---|---|---|
| When all dose groups are used | 0.12 | 1.23 x $10^4$ | 0 | 0 | 1.67 x $10^4$ | 6.67 (d.f. = 2)<br>$0.025 < p < 0.05$ |
| When the highest dose group is not used | | | Above fit is satisfactory | | | |
| | 0.09 | 0 | 3.5 x $10^9$ | 0 | 4.69 x $10^4$ | 0.92 (d.f. = 1)<br>$p > 0.25$ |
| When the two highest dose groups are not used | | | Above fit is satisfactory | | | |

$_aq_1^*$ = the maximum linear component from the model with adequate goodness of fit ($p > 0.01$) = $1.67 \times 10^4 - 4.69 \times 10^4$ $(mg/kg/day)^{-1}$

$q_1^*$ = $_aq_1^*$ $(70/w_a)^{1/3}$ = $8.98 \times 10^4 - 2.52 \times 10^5$ $(mg/kg/day)^{-1}$, the upper 95% limit slope factor associated with human dose response depending on inclusion or exclusion of the highest dose data.

## TABLE B-8A

### Curve Fit of the Multistage Model Parameters to Experimental Data by Study and Pathologist
### Linear Parameter $q_1$, Maximized to Give Upper 95% Limit $q_1^*$

Compound......................2,3,7,8-TCDD
Study.........................Dow
Sex-species...................Female rat
Weight ($w_a$)..................450 g
Tumor sites (one or more)....Liver, lung, hard palate, or nasal turbinates (ref. Table B-2)

Pathologist - Kociba (Eliminating first year's data to adjust for high early mortality in the high-dose group.)

| Exposure level (mg/kg/day) | 0 | $1 \times 10^{-6}$ | $1 \times 10^{-5}$ | $1 \times 10^{-4}$ |
|---|---|---|---|---|
| +r/n | 9/85 | 3/48 | 18/48 | 34/40 |

+r = number of animals with one or more of the tumors
n = total number of animals examined

| Estimated multistage parameters | $q_0$ | $q_1$ | $q_2$ | $q_3$ | $_aq_1^*$ | Goodness of fit $X^2$ |
|---|---|---|---|---|---|---|
| When all dose groups are used | 0.11 | $2.08 \times 10^4$ | 0 | 0 | $2.82 \times 10^4$ | 3.38 (d.f. = 2)<br>$0.25 < p < 0.10$ |
| When the highest dose group is not used | | | Above fit is satisfactory | | | $p > 0.25$ |

$_aq_1^*$ = the maximum linear component from the model with adequate goodness of fit ($p>0.01$) = $2.82\times10^4$ (mg/kg/day)$^{-1}$

$q_1^* = {_aq_1^*}\ (70/w_a)^{1/3} = 1.51\times10^5$ (mg/kg/day)$^{-1}$, the upper 95% limit slope factor associated with human dose response.

## TABLE B-9

### Curve Fit of the Multistage Model Parameters to Experimental Data by Study and Pathologist Linear Parameter $q_1$, Maximized to Give Upper 95% Limit $q_1$*

Compound.....................2,3,7,8-TCDD
Study........................Kociba - Dow
Sex-species..................Female rat
Weight ($w_a$)..................450 g
Tumor sites (one or more)....Liver, lung, hard palate, or nasal turbinates (ref. Table B-2)

Pathologist - Squire

| Exposure level (mg/kg/day) | 0 | $1 \times 10^{-6}$ | $1 \times 10^{-5}$ | $1 \times 10^{-4}$ |
|---|---|---|---|---|
| +r/n | 16/86 | 8/50 | 27/50 | 34/47 |

+r = number of animals with one or more of the tumors
n = total number of animals examined

| Estimated multistage parameters | $q_0$ | $q_1$ | $q_2$ | $q_3$ | $_aq_1$* | Goodness of fit $X^2$ |
|---|---|---|---|---|---|---|
| When all dose groups are used | 0.26 | $1.25 \times 10^4$ | 0 | 0 | | 9.8 (d.f. = 2) $p<0.01$ |
| When the highest dose group is not used | 0.19 | 0 | $5.83 \times 10^9$ | | $7.90 \times 10^4$ | 0.209 (d.f. = 1) |
| When the two highest dose groups are not used | | | Above fit is satisfactory | | | |

$_aq_1$* = the maximum linear component from the model with adequate goodness of fit ($p>0.01$) = $7.90 \times 10^4$ $(mg/kg/day)^{-1}$

$q_1$* = $_aq_1$* $(70/w_a)^{1/3}$ = $4.25 \times 10^5$ $(mg/kg/day)^{-1}$, the upper 95% limit slope factor associated with human dose response.

## TABLE B-9A

## Curve Fit of the Multistage Model Parameters to Experimental Data by Study and Pathologist Linear Parameter $q_1$, Maximized to Give Upper 95% Limit $q_1^*$

Compound....................2,3,7,8-TCDD
Study.......................Kociba - Dow
Sex-species.................Female rat
Weight ($w_a$)..................450 g
Tumor sites (one or more)....Liver, lung, hard palate, or nasal turbinates (ref. Table B-2)

Pathologist - Squire (Eliminating first year's data to adjust for high early mortality in the high-dose group.)

| Exposure level (mg/kg/day) | 0 | $1 \times 10^{-6}$ | $1 \times 10^{-5}$ | $1 \times 10^{-4}$ |
|---|---|---|---|---|
| +r/n | 16/85 | 8/48 | 27/48 | 34/40 |

+r = number of animals with one or more of the tumors
n = total number of animals examined

| Estimated multistage parameters | $q_0$ | $q_1$ | $q_2$ | $q_3$ | $_aq_1^*$ | Goodness of fit $X^2$ |
|---|---|---|---|---|---|---|
| When all dose groups are used | 0.24 | $2.12 \times 10^4$ | 0 | 0 | $3.00 \times 10^4$ | 6.41 (d.f.= 2)<br>$0.025 < p < 0.05$ |
| When the highest dose group is not used | | | Above fit is satisfactory | | | |

$_aq_1^*$ = the maximum linear component from the model with adequate goodness of fit ($p>0.01$) = $3.00 \times 10^4$ $(mg/kg/day)^{-1}$

$q_1^* = {}_aq_1^* \; (70/w_a)^{1/3} = 1.61 \times 10^5 \; (mg/kg/day)^{-1}$, the upper 95% limit slope factor associated with human dose response.

## TABLE B-10

### Curve Fit of the Multistage Model Parameters to Experimental Data by Study and Pathologist Linear Parameter $q_1$, Maximized to Give Upper 95% Limit $q_1^*$

Compound......................2,3,7,8-TCDD
Study.........................NCI
Sex-species...................Female rat
Weight ($w_a$)...................450 g
Tumor sites (one or more)....Liver neoplastic nodules or hepatocellular carcinoma (ref. Table B-3)

Pathologist - NCI Reviewed

| Exposure level (mg/kg/day) | 0 | $1.43 \times 10^{-6}$ | $7.14 \times 10^{-6}$ | $7.14 \times 10^{-5}$ |
|---|---|---|---|---|
| +r/n | 5/75 | 1/49 | 3/50 | 14/49 |

+r = number of animals with one or more of the tumors
n = total number of animals examined

| Estimated multistage parameters | $q_0$ | $q_1$ | $q_2$ | $q_3$ | $_aq_1^*$ | Goodness of fit $X^2$ |
|---|---|---|---|---|---|---|
| When all dose groups are used | 0.05 | 0 | $5.65 \times 10^7$ | 0 | $6.09 \times 10^3$ | 1.44 (d.f. = 2) |
| When the highest dose group is not used | Above fit is satisfactory | | | | | |

$_aq_1^*$ = the maximum linear component from the model with adequate goodness of fit ($p>0.01$) = $6.09 \times 10^3$ $(mg/kg/day)^{-1}$

$q_1^*$ = $_aq_1^* \ (70/w_a)^{1/3}$ = $3.28 \times 10^4$ $(mg/kg/day)^{-1}$, the upper 95% limit slope factor associated with human dose response.

## TABLE B-11

### Curve Fit of the Multistage Model Parameters to Experimental Data by Study and Pathologist Linear Parameter $q_1$, Maximized to Give Upper 95% Limit $q_1^*$

Compound.....................2,3,7,8-TCDD
Study........................NCI
Sex-species..................Male mice
Weight ($w_a$)..................48 g
Tumor sites (one or more)....Hepatocellular carcinomas (ref. Table B-4)

Pathologist - NCI Review

| Exposure level (mg/kg/day) | 0 | $1.43 \times 10^{-6}$ | $7.14 \times 10^{-6}$ | $7.14 \times 10^{-5}$ |
|---|---|---|---|---|
| +r/n | 8/73 | 9/49 | 8/49 | 17/50 |

+r = number of animals with one or more of the tumors
n = total number of animals examined

| Estimated multistage parameters | $q_0$ | $q_1$ | $q_2$ | $q_3$ | $_aq_1^*$ | Goodness of fit $X^2$ |
|---|---|---|---|---|---|---|
| When all dose groups are used | 0.15 | $3.80 \times 10^{3}$ | 0 | 0 | $6.63 \times 10^{3}$ | 2.43 (d.f. = 2) |
| When the highest dose group is not used | | | Above fit is satisfactory | | | |

$_aq_1^*$ = the maximum linear component from the model with adequate goodness of fit ($p>0.01$) = $6.63 \times 10^{3}$ $(mg/kg/day)^{-1}$

$q_1^*$ = $_aq_1^* \ (70/w_a)^{1/3} = 7.52 \times 10^{4}$ $(mg/kg/day)^{-1}$, the upper 95% limit slope factor associated with human dose response.

## TABLE B-12

## Curve Fit of the Multistage Model Parameters to Experimental Data by Study and Pathologist Linear Parameter $q_1$, Maximized to Give Upper 95% Limit $q_1^*$

Compound.....................2,3,7,8-TCDD
Study........................NCI
Sex-species..................Female mice
Weight ($w_a$)..................40 g
Tumor sites (one or more)....Subcutaneous tissue - fibrosarcoma, hematopoietic system lymphoma, or leukemia
Liver - hepatocellular adenoma or carcinoma (ref. Table B-5)

Pathologist - NCI Reviewed

| Exposure level (mg/kg/day) | 0 | $5.71 \times 10^{-6}$ | $2.86 \times 10^{-5}$ | $2.86 \times 10^{-4}$ |
|---|---|---|---|---|
| +r/n | 22/74 | 20/50 | 19/48 | 31/47 |

+r = number of animals with one or more of the tumors
n = total number of animals examined

| Estimated multistage parameters | $q_0$ | $q_1$ | $q_2$ | $q_3$ | $_aq_1^*$ | Goodness of fit $\chi^2$ |
|---|---|---|---|---|---|---|
| When all dose groups are used | 0.41 | $2.38 \times 10^3$ | 0 | 0 | $3.78 \times 10^3$ | 1.20 (d.f. = 2) |
| When the highest dose group is not used | Above fit is satisfactory | | | | | |

$_aq_1^*$ = the maximum linear component from the model with adequate goodness of fit ($p>0.01$) = $3.78\times10^3$ $(\text{mg/kg/day})^{-1}$

$q_1^*$ = $_aq_1^*\ (70/w_a)^{1/3}$ = $4.56\times10^4$ $(\text{mg/kg/day})^{-1}$, the upper 95% limit slope factor associated with human dose response.

## TABLE B-13

### Summary of Human Slope Estimates for 2,3,7,8-TCDD

| Species | Study | Sex | Pathologist | Human Slope Estimate $q_1^*$ in $(mg/kg/day)^{-1}$ | Ref. Table No. |
|---|---|---|---|---|---|
| Rat | Dow | Male | Kociba | $1.47 \times 10^4$ | B6 |
| Rat | | | Squire | $1.73 \times 10^4$ | B7 |
| Rat | | Female | Kociba - unadjusted | $8.98 \times 10^4 - 2.52 \times 10^5$ | B8 |
| | | | - adjusted for early deaths | $1.51 \times 10^5$† | B8A |
| Rat | | Female | Squire - unadjusted | $4.25 \times 10^5$ | B9 |
| | | | - adjusted for early deaths | $1.61 \times 10^5$† | B9A |
| Rat | NCI | Female | NCI - Reviewed | $3.28 \times 10^4$ | B10 |
| Mice | NCI | Male | NCI - Reviewed | $7.52 \times 10^4$ | B11 |
| Mice | | Female | NCI - Reviewed | $4.56 \times 10^4$ | B12 |

†Values used to determine geometric mean of $1.56 \times 10^5$ $(mg/kg/day)^{-1}$

# APPENDIX C

## COMPARISON OF RESULTS BY VARIOUS EXTRAPOLATION MODELS

The estimate of unit risk from animals presented in the body of this document is calculated by the use of the linearized multistage model, for the reasons given herein. The use of this nonthreshold model is part of a methodology that estimates a conservative linear slope at low extrapolation doses that is usually consistent with the data at all dose levels in an experiment. The model holds that the most plausible upper limits of risk are those predicted by linear extrapolation to low levels of the dose-response relationship.

Other nonthreshold models that have been used for risk extrapolation are the one-hit, the log-Probit, and the Weibull models. The one-hit model is characterized by a continuous downward curvature, but is linear at low doses. Because of its functional form, the one-hit model can be considered the linear form or first stage of the multistage model. This fact, together with the downward curvature of the one-hit model, means that it will always yield low-level risk estimates which are at least as large as those of the multistage model. In addition, whenever the data can be fitted adequately by the one-hit model, estimates based on the one-hit model and the multistage model will be comparable.

The log-Probit and the Weibull models, because of their general "S" curvature, are often used for the interpretation of toxicological data in the observable range. The low-dose upward curvatures of these two models usually yield lower low-dose risk estimates than those of the one-hit or multistage models. The log-Probit model was originally used in biological assay problems such as potency assessments of toxicants and drugs, and is generally used to estimate such values as percentile lethal dose or percentile effective dose. The development of the model occurred along strictly empirical lines, i.e., it was observed in these studies that several log dose-response relationships followed the cumulative normal probability distribution function, $\Phi$. In fitting the cancer bioassay data, assuming an independent background, this becomes

$$P(D;a,b,c) = c + (1-c)\,\Phi\,(a+b\log_{10} D) \quad a,b > 0 < c < 1$$

where P is the proportion responding at dose D, c is an estimate of the background rate, a is an estimate of the standardized mean of individual tolerances, and b is an estimate of the log dose-Probit response slope.

The one-hit model arises from the theory that a single molecule of a carcinogen has a probability of transforming a single normal cell into a cancer cell. It has the probability distribution function

$$P(D;a,b) = 1-\exp-(a+bd) \quad a,b > 0$$

where a and b are the parameter estimates. The estimate a represents the background or zero dose rate, and the parameter estimated by b represents the linear component or slope of the dose-response model. In discussing the added risk over background, incorporation of Abbott's correction leads to

$$P(D;b) = 1-\exp-(bd) \quad b > 0$$

Finally, a model from the theory of carcinogenesis arises from the multihit model applied to multiple target cells. This model has been termed here the Weibull model. It is of the form

$$P(D;b,k) = 1-\exp-(bd^{k}) \quad b,k > 0$$

For the power of dose only, the restriction $k > 0$ has been placed on this model. When $k > 1$, this model yields low-dose estimates of risks usually significantly lower than either the multistage or one-hit models, which are linear at low doses. When $0 < k < 1$, the model yields low-dose estimates of risk that are greater than the one-hit and multistage models; this is generally regarded as biologically implausible. All three of these models usually project risk estimates that are significantly higher at low exposure levels than those projected by the log-Probit model.

The Dow Chemical Company data for female Sprague-Dawley rats were fitted to the above models, after adjusting for early mortality by eliminating all animals dying before 1 year. The results are identical for the multistage and one-hit models, as shown in Tables C-1 and C-2. The log-Probit model yielded by far the lowest estimates at low doses. The Weibull model yielded estimates higher (by two orders of magnitude) at low levels than either the one-hit or the multistage model, since k, determined by best fit to the data, is <1. As discussed in the text and shown in Tables B-8 and B-9, dropping the highest dose resulted in a larger upper-limit slope estimate for the multistage model. However, without the highest dose points, neither the log-Probit nor the Weibull models could be fitted to the data, for the reason that the control group response was higher than that of the lowest dose group.

## TABLE C-1

Estimates of Low-Dose Risk to Humans Exposed to 2,3,7,8-TCDD Based on Female Sprague-Dawley Rats From the Dow Chemical Co. Feeding Study Derived from Four Different Models Data - Kociba Analysis, Adjusting for Early Mortality (Ref. Table B-8A)

| | Maximum Likelihood Estimates of Additional Risks | | | 95% Upper Confidence Limit of Additional Risks | | |
|---|---|---|---|---|---|---|
| Dose (ng/kg/day) | Multistage/One-hit Model Model[a] | Weibull Model[b] | Log-Probit Model | Multistage/One-hit Model Model* | Weibull Model | Log-Probit Model |
| $10^{-5}$ | $1.1 \times 10^{-6}$ | $1.8 \times 10^{-5}$ | 0 | $1.5 \times 10^{-6}$ | $9.7 \times 10^{-5}$ | $7.7 \times 10^{-10}$ |
| $10^{-4}$ | $1.1 \times 10^{-5}$ | $1.1 \times 10^{-4}$ | $1.2 \times 10^{-13}$ | $1.5 \times 10^{-5}$ | $5.3 \times 10^{-4}$ | $3.0 \times 10^{-12}$ |
| $10^{-3}$ | $1.1 \times 10^{-4}$ | $7.1 \times 10^{-4}$ | $4.9 \times 10^{-9}$ | $1.5 \times 10^{-4}$ | $2.9 \times 10^{-3}$ | $7.5 \times 10^{-8}$ |
| $10^{-2}$ | $1.1 \times 10^{-3}$ | $4.5 \times 10^{-3}$ | $1.7 \times 10^{-5}$ | $1.5 \times 10^{-3}$ | $1.5 \times 10^{-2}$ | $1.5 \times 10^{-4}$ |
| $10^{-1}$ | $1.1 \times 10^{-2}$ | $2.9 \times 10^{-2}$ | $5.2 \times 10^{-3}$ | $1.5 \times 10^{-2}$ | $7.2 \times 10^{-2}$ | $2.3 \times 10^{-2}$ |
| 1 | $1.1 \times 10^{-1}$ | $1.7 \times 10^{-1}$ | $1.7 \times 10^{-1}$ | $1.4 \times 10^{-1}$ | $3.0 \times 10^{-1}$ | $3.1 \times 10^{-1}$ |

[a] Both models gave identical results

[b] The value of k, determined by best fit to the data, is <1.

| | | | | |
|---|---|---|---|---|
| Human equivalent dose (ng/kg/day): | 0 | 0.186 | 1.86 | 18.6 |
| Animal tumors/number examined: | 9/85 | 3/48 | 18/48 | 34/40 |

Human equivalence conversion: 1 ng/kg/day (oral) = 25.0 ng/m³ in air

## TABLE C-2

Estimates of Low-Dose Risk to Humans Exposed to 2,3,7,8-TCDD Based on Female Sprague-Dawley Rats From the Dow Chemical Co. Feeding Study Derived from Four Different Models Data - Squire Analysis, Adjusting for Early Mortality (Ref. Table B-9A)

| Dose (ng/kg/day) | Maximum Likelihood Estimates of Additional Risks |  |  | 95% Upper Confidence Limit of Additional Risks |  |  |
|---|---|---|---|---|---|---|
|  | Multistage/One-hit Model Model[a] | Weibull Model[b] | Log-Probit Model | Multistage/One-hit Model Model* | Weibull Model | Log-Probit Model |
| $10^{-5}$ | $1.1\times10^{-6}$ | $3.0\times10^{-4}$ | $2.2\times10^{-12}$ | $1.6\times10^{-6}$ | $1.3\times10^{-3}$ | $4.4\times10^{-10}$ |
| $10^{-4}$ | $1.1\times10^{-5}$ | $1.2\times10^{-3}$ | $7.6\times10^{-9}$ | $1.6\times10^{-5}$ | $4.4\times10^{-3}$ | $1.1\times10^{-7}$ |
| $10^{-3}$ | $1.1\times10^{-4}$ | $4.9\times10^{-3}$ | $5.8\times10^{-5}$ | $1.6\times10^{-4}$ | $1.5\times10^{-2}$ | $5.3\times10^{-5}$ |
| $10^{-2}$ | $1.1\times10^{-3}$ | $2.0\times10^{-2}$ | $9.2\times10^{-4}$ | $1.6\times10^{-3}$ | $5.1\times10^{-2}$ | $5.8\times10^{-3}$ |
| $10^{-1}$ | $1.1\times10^{-2}$ | $7.8\times10^{-2}$ | $3.3\times10^{-2}$ | $1.6\times10^{-2}$ | $1.6\times10^{-1}$ | $1.1\times10^{-1}$ |
| 1 | $1.1\times10^{-1}$ | $2.8\times10^{-1}$ | $2.9\times10^{-1}$ | $1.5\times10^{-1}$ | $4.3\times10^{-1}$ | $4.8\times10^{-1}$ |

[a] Both models gave identical results

[b] The value of k, determined by best fit to the data, is <1.

|  |  |  |  |  |
|---|---|---|---|---|
| Human equivalent dose (ng/kg/day): | 0 | 0.186 | 1.86 | 18.6 |
| Animal tumors/number examined: | 16/85 | 8/48 | 27/48 | 34/40 |

Human equivalence conversion: 1 ng/kg/day (oral) = 25.0 ng/m³ in air

# APPENDIX D

A toxicity-based risk assessment has been calculated for comparison with the cancer-based risk assessment in accordance with the recommendations by U.S. EPA's Science Advisory Board.

2,3,7,8-TCDD is an unusually toxic compound with demonstrated acute, subacute and chronic effects in animals and humans. Acute or subchronic exposures to 2,3,7,8-TCDD can adversely affect the skin, the liver, the nervous system and the immune system.

2,3,7,8-TCDD displays an unusually high degree of reproductive toxicity. It is teratogenic, fetotoxic and reduces fertility. In a three-generation reproductive study, Murray et al. (1979) reported a reduction in fertility after daily dosing at 0.1 or 0.01 μg 2,3,7,8-TCDD/kg in the $F_1$ and $F_2$ generations of Sprague-Dawley rats. Although Murray et al. (1979) considered the lowest dose tested, 0.001 μg/kg, to be a NOEL, a re-evaluation of these data by Nisbet and Paxton (1982), using different statistical methods, indicated that there was a reduction in the gestation index, decreased fetal weight, increased liver to body weight ratio, and increased incidence of dilated renal pelvis at the 0.001 μg/kg dose. The re-evaluated data would suggest that equivocal adverse effects were seen at the lowest dose (0.001 μg/kg/day) and that this dose should, therefore, represent a LOAEL. Schantz et al. (1979) found reductions in fertility and various other toxic effects in rhesus monkeys fed a 50 ppt 2,3,7,8-TCDD diet for 20 months. This corresponds to a calculated daily dose of 0.0015 μg 2,3,7,8-TCDD/kg/day. These results suggest that monkeys may be somewhat more sensitive than rats, since the effects in monkeys were more severe and not equivocal.

A toxicity-based criterion has been calculated for comparison with the cancer-based criterion in accordance with public comments. Since the data from the limited study by Schantz et al. (1979) are supportive of the findings by Murray et al. (1979), it seems reasonable to determine an ADI based on the LOAEL. If one selects an uncertainty factor of 100 based on the existence of lifetime animal studies and knowledge of effects in man as per U.S. EPA methodologies (Federal Register, 1980b), and then an additional 10 because a LOAEL is used as the basis of this calculation,* then the ADI for a 70 kg man would be:

$$\text{ADI} = \frac{10^{-3}\ \mu\text{g/kg/day (LOAEL)}}{100 \times 10} = 7.0 \times 10^{-5}\ \mu\text{g/kg/day.}$$

However, this concentration may not be sufficiently protective of human health since it does not take into account the demonstrated carcinogenic effects of 2,3,7,8-TCDD in animals and the probability that 2,3,7,8-TCDD is a human carcinogen as discussed in Section 11.6.1.

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*According to the methods published by U.S. EPA (Federal Register, 1980b), an additional uncertainty factor between 1 and 10 must be used because the calculation is based on a LOAEL. An uncertainty factor of 10 was chosen because of the adverse effects seen in rhesus monkeys at 0.0015 µg/kg/day, despite the equivocal nature of the effects in rats seen at the 0.001 µg/kg/day dose level.

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